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Research Article

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Vasoactive Mediators as the "Trigger Mechanism" of Endotoxin Shock *

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The mechanisms by which endotoxin induces a profound shock state have not been clearly established. Several neurohumoral agents have been implicated as mediators of endotoxin shock. These include histamine (1–4), catecholamines (5–7), and serotonin (7–9).

Inferences of the role played by any of these mediators have been derived from four types of evidence: *a*) the hemodynamic alterations observed in endotoxemia are simulated by vascular effects of the naturally occurring substance (4, 5), *b*) plasma concentrations of the neurohumoral agent undergo changes in endotoxin shock (7, 10–13), *c*) vascular reactivity to the agent is altered in endotoxemia (5, 6, 14), and *d*) pharmacological antagonists to the substance in question prevent certain responses to endotoxin (4, 15–17).

That a primary role could be assigned to any single substance in endotoxin shock is doubtful, however, because of the complexity of endotoxemia and the frequently conflicting and occasionally inconclusive nature of the evidence (18). Our investigation was prompted by the uncertainties concerning the relative importance of several proposed intermediaries in the early phase of endotoxin shock. Experiments were designed to utilize some of the approaches mentioned above.

Methods

Studies of endotoxin shock were performed in 71 dogs of both sexes weighing 10 to 20 kg each. All animals were anesthetized with pentobarbital sodium (30 mg per kg). These experiments may be divided into three types: *a*) those in which endotoxemia was induced in animals pretreated to deplete tissue supplies of histamine, catecholamines, or serotonin; *b*) studies in which the hemodynamic and chemical effects of infused histamine, catecholamines, acetylcholine, or serotonin were compared with the effects of endotoxin injection; and *c*) experiments in which vascular sensitivity to histamine, catecholamines, or serotonin was determined at various times before and after injecting endotoxin. The design of these experiments is outlined in Table I.

Mean pressures in millimeters of Hc were monitored in arteries, veins, or perfusion circuits by pressure transducers and recorded on an oscillograph.[1] Plasma concentrations of epinephrine and norepinephrine (micrograms per liter) were determined by the method of Weil-Malherbe and Bone (19) as modified in this laboratory (20). Platelet-free plasma concentrations of serotonin were determined by the method of Waalkes (21) using an interim wash with salt-saturated NaOH to remove residual traces of histidine. After the final acid extraction one sample was employed for the assay of serotonin; another sample was used for the fluorometric determination of histamine (22).

Recoveries with these methods are as follows: catecholamines, 96 to 98% with values exceeding 1 μg per L and 75% with lesser values; serotonin, approximately 100%; histamine, 90 to 100%. The sensitivities of the chemical analyses are these: epinephrine, 0.6 μg per L; norepinephrine, 1 μg per L; serotonin, 5 μg per L; and histamine, 2 μg per L.

One lot of the endotoxin of *Escherichia coli*, 0–111,[2] was used in all these experiments. A sublethal dose (an approximate LD_{50}) of 0.8 mg per kg was injected into a femoral vein to induce endotoxemia. To avoid possible nonspecific responses and masking of the effects of pharmacological agents, a sublethal amount was selected rather than the more commonly reported doses that are many times an LD_{100}.

Experiments and Results

a) Control experiments

Heparin sodium (10 mg per kg) was administered to all dogs used for control (groups C and PV) and depletion (groups F and R) experiments. In 5 dogs (group C) blood samples were obtained from the right femoral artery at 30

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[1] Sanborn Co., Waltham, Mass.

[2] Difco Laboratories, Detroit, Mich.

TABLE I

*Design of experiments comprising this study**

Group	No. dogs	Treatment before endotoxin	Measurements
a. Control experiments			
C	5	None	SP, arterial H, E, N, S
PV	3	None	SP, arterial H, E, N, S MV, portal H, E, N, S
b. Depletion experiments			
F	5	Compound 48/80 ± cortisone acetate	SP, arterial H, E, N, S
R	5	Reserpine	SP, arterial H, E, N, S
c. Infusion experiments			
H-1	5	Histamine in jugular vein	SP, MP, LP, LV, MV, and arterial H, E, N, S
H-2	5	Histamine in jugular and mesenteric veins	SP, MP, LP, LV, MV
A-1	3	Acetylcholine in jugular vein	SP, MP, LP, LV, MV, and arterial H, E, N, S
A-2	5	Acetylcholine in jugular and mesenteric veins	SP, MP, LP, LV, MV
EN-1	3	Epinephrine in jugular vein	SP, MP, LP, LV, MV, and arterial H, E, N, S
EN-2	3	Epinephrine in jugular vein and norepinephrine in mesenteric vein	SP, MP, LP, LV, MV
S	5	Serotonin infused with endotoxin	SP, MP, LP, LV, MV, and arterial H, E, N, S
d. Sensitivity experiments			
AH	3	Histamine effect on SP	SP
PH	3	Histamine effect on MV	MV
AN	3	Norepinephrine effect on SP	SP
PN	3	Norepinephrine effect on MV	MV
AE	3	Epinephrine effect on SP	SP
PE	3	Epinephrine effect on MV	MV
AS	3	Serotonin effect on SP	SP
PS	3	Serotonin effect on MV	MV

* Abbreviations in this and subsequent tables: SP = systemic arterial pressure, H = histamine concentration, E = epinephrine concentration, N = norepinephrine concentration, S = serotonin concentration, MV = mesenteric venous pressure, MP = perfused mesenteric arterial pressure, LP = perfused femoral arterial pressure, and LV = femoral venous pressure.

minutes and immediately before injecting endotoxin, and at 2 minutes, 30 minutes, and 5 hours after endotoxin. Plasma concentrations of histamine (H), epinephrine (E), norepinephrine (N), and serotonin (S) were determined from these samples. Systemic arterial pressures were monitored at the times mentioned as well as at hourly intervals after the injection of endotoxin.

In these 5 animals (group C) endotoxin induced within a few minutes an abrupt profound fall in systemic arterial pressure (SP) that never returned to preinjection levels in any dog over the subsequent 5 hours of observation. These results are shown in Table II.

Plasma E increased from preinjection levels at 5 minutes after endotoxin in 4 of these dogs and increased further at 30 minutes and 5 hours after endotoxin. Plasma S concentrations in all 5 dogs were markedly decreased at 5 minutes and were still below preinjection levels 30 minutes and 5 hours later. There were no consistent effects upon either plasma H or N concentrations in these dogs. These results appear in Table II.

In 3 animals (group PV) a left subcostal laparotomy was performed and a cannula inserted into a splenic vein. Femoral arterial and portal venous blood samples were obtained and vascular pressures measured before injecting endotoxin

TABLE II

*Effects of endotoxin on pressures and neurohumoral substances in the systemic arterial and portal venous circulations of 2 groups of control dogs (C and PV)**

		Time, *minutes*								
		−30	−5	+2	+30	+60	+120	+180	+240	+300
Group C										
	SP		156±6	52±8	100±8	90±8	114±16	126±14	118±12	104±18
Arterial	H	18±2	21±3	16±3	19±3					20±3
Arterial	E	0.3±0.2	0.4±0.2	1.9±0.6	2.6±0.6					11.2±6.8
Arterial	N	1.5±0.3	1.5±0.2	2.6±1.3	2.0±0.4					3.0±0.9
Arterial	S	293±54	311±79	25±4	100±23					218±78
			Time, *minutes*							
			−5	+5	+10	+15	+20	+25	+30	
Group PV										
	SP		152±13	65±18	87±15	87±9	98±10	105±10	113±10	
Arterial	H		19.7±0.9	19.1±1.9	18.3±0.8				16.6±2.0	
Arterial	E		0.8±0.3	10.3±8.3	6.6±2.1				2.7±0.7	
Arterial	N		1.4±0.4	2.5±0.7	1.9±0.5				1.8±0.2	
Arterial	S		395±27	90±27	126±54				129±35	
	MV		9±1	23±4	14±2	10±2	9±2	8±1	8±1	
Portal	H		19.9±1.7	19.6±2.9	20.8±0.7				17.7±1.1	
Portal	E		0.7±0.3	5.9±0.7	3.7±0.6				2.4±0.5	
Portal	N		3.2±1.2	13.9±9.8	10.4±5.9				7.0±5.2	
Portal	S		419±41	141±52	133±51				139±34	

* Abbreviations as in Table I. In this and all subsequent tables, times are in relation to the injection of endotoxin. In this and all subsequent tables, pressures are in millimeters of Hg and chemical concentrations in micrograms per liter plasma (to the nearest whole number in some tables). All values represent the mean ± standard error of the mean (SEM) for each group of 3 or 5 dogs at that time.

and at 5, 10, and 30 minutes after injection. Plasma H, E, N, and S concentrations were determined from the separate samples.

In these animals, the same changes were noted as in group C, namely, a sudden decline in SP and systemic arterial S and an increase in arterial E with no change in N or H. Portal venous responses to endotoxin included an abrupt increase in portal venous pressure (MV) maximal at 5 minutes after injection and gradually returning to preinjection pressures, a fall in portal venous S, a rise in both E and N at 10 and 30 minutes, and no effect on H. These results also appear in Table II.

b) Depletion experiments

Compound 48/80 [3] was administered to 5 dogs (group F) according to the following schedule: 0.2 mg per kg was injected into the peritoneum twice daily for one day, 0.5 mg per kg was injected twice on a second day, and 1.0 mg per kg was injected on a third day 2 hours before administering endotoxin. Cortisone acetate (200 mg) was injected intramuscularly each day for 3 days before endotoxin in 3 of these dogs to reduce further the tissue stores of histamine (23). Plasma H, E, N, or S concentrations and femoral arterial pressures were obtained at the same time intervals after the injection of endotoxin as has been described for the control dogs (group C).

These animals (group F) responded to endotoxin in a manner similar to controls: an abrupt decline in SP and S, an increase in E, and a slight increase in N and H at 2 minutes after endotoxin. These results are detailed in Table III.

Reserpine [4] (0.1 mg per kg) was injected subcutaneously twice daily for 4 days in 5 dogs (group R) before injecting endotoxin. SP and plasma H, E, N, and S values were determined as in the C and F groups of animals.

These animals were adversely affected by reserpine. Several dogs exhibited a bloody diarrhea, lethargy, and anorexia, and all exhibited a lower starting SP. Furthermore, 3 of the animals died within 2½ hours after the administration of endotoxin. Endotoxin induced a profound fall in SP that remained lower throughout the observation

[3] Burroughs, Wellcome and Co., Inc., Tuckahoe, N. Y.

[4] Ciba Pharmaceutical Co., Summit, N. J.

TABLE III

*Effects of endotoxin on pressures and neurohumoral substances in animals pretreated with compound 48/80 ± cortisone (group F) or reserpine (group R)**

	Time, *minutes*								
	−30	−5	+2	+30	+60	+120	+180	+240	+300
Group F									
SP		160±16	58±12	108±14	86±16	90±14	106±16	98±14	104±16
Arterial H	23±4	23±4	29±3	26±3					25±7
Arterial E	0.5±0.3	0.5±0.2	6.6±3.1	1.7±0.7					2.3±0.8
Arterial N	1.8±0.3	1.5±0.3	2.3±0.4	2.0±0.2					2.4±0.3
Arterial S	503±90	421±121	61±23	165±30					274±85
Group R									
SP		142±10	34±6	78±10	76±10	56±10	82±18	68±12	52±4
Arterial H	28±6	25±4	29±3	24±2					34+10
Arterial E	0.3±0.1	0.2±0.2	0.8±0.5	1.6±0.5					1.5±0.6
Arterial N	1.2±0.2	2.2±1.1	1.7±0.3	1.6±0.3					2.2±0.0
Arterial S	21±6	21±5	21±6	29±7					47±23

*All values represent mean ± SEM obtained from 5 dogs at the specified time.

period than SP values in any preceding group (Table III). Plasma S was markedly reduced by reserpine and did not fall further after endotoxin. Plasma E increased but H and N were unaffected.

c) Infusion experiments

Heparin was also administered to these dogs. An endotracheal tube was inserted and connected to a positive pressure respirator with a respiratory minute volume of 2.5 to 4.0 L that maintained a normal pH and Po_2. The superior mesenteric artery was exposed through a left subcostal laporatomy, and the vessel was cannulated and perfused at a constant rate by a finger pump [5] with an inlet tubing connected to a centrally directed cannula in the right femoral artery. A flow was selected that yielded a mean pressure comparable to systemic arterial pressure. In 29 experiments flow through this perfusion circuit averaged 126 ± 11 (SE) ml per minute, which agrees with previous reports (24, 25) for this type of perfusion. A second finger pump was interposed between the left common carotid and left femoral arteries, and the left hind limb was perfused at flows providing pressures comparable to systemic arterial pressure (26 ± 3 ml per minute). Pressures were monitored in a nonperfused artery, in both perfusion circuits, and in the femoral and mesenteric veins. In many of these animals arterial blood samples were obtained for the determination of plasma H, E, N, and S concentrations at various time intervals after the infusion of one of several neurohumoral agents and endotoxin.

1) Histamine. In 10 dogs of this series, hemodynamic changes were observed, and alterations in plasma H, E, N, and S were measured during the infusion of histamine and subsequently in response to endotoxin. Histamine [6] was infused for 30 minutes into the jugular vein of 5 of these dogs (group H-1) in amounts (5 to 14 μg histamine base per minute) that induced a fall in systemic pressure (SP) comparable to the hypotension observed in control dogs given endotoxin. In the other 5 dogs of this group (group H-2), simultaneous infusions of histamine were maintained in both the portal circulation (10 to 25 μg per minute for 5 to 10 minutes) and in a systemic vein (5 to 25 μg per minute for 20 to 30 minutes) to induce both a fall in SP and an increase in mesenteric venous pressure (MV). Pressures were monitored every 5 minutes for 30 minutes after starting the infusion of histamine in a nonperfused systemic artery (SP), the perfused mesenteric artery (MP) and femoral artery (LP), the left femoral vein (LV), and a mesenteric vein (MV) in all dogs receiving histamine. In the 5 dogs in which histamine was infused systemically only (group H-1), plasma H, E, N, and S levels were determined before infusing histamine and at 5, 10, and 30 minutes after beginning the drug.

[5] Sigmamotor Co., Middleport, N. Y.

[6] Histamine acid phosphate, Eli Lilly and Co., Indianapolis, Ind.

TABLE IV

*Effects of systemically infused histamine and of endotoxin on hemodynamic and neurohumoral parameters (group H-1)**

	Time, *minutes*						
	−5	+5	+10	+15	+20	+25	+30
	Systemic infusion of histamine						
SP	107 ± 9	98 ± 8	84 ± 9	70 ± 10	70 ± 12	71 ± 11	75 ± 11
MP	104 ± 14	114 ± 15	121 ± 16	119 ± 17	118 ± 16	116 ± 15	114 ± 14
LP	116 ± 8	112 ± 5	108 ± 7	106 ± 10	106 ± 12	110 ± 13	108 ± 14
LV	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1
MV	7 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0
H	24 ± 3	25 ± 5	32 ± 7				30 ± 5
E	3 ± 0	6 ± 1	10 ± 1				9 ± 2
N	2 ± 1	2 ± 1	2 ± 0				2 ± 0
S	157 ± 56	190 ± 69	148 ± 55				161 ± 50
	Injection of endotoxin						
SP	76 ± 9	46 ± 12	42 ± 9	42 ± 5	38 ± 8	44 ± 8	44 ± 9
MP	105 ± 12	128 ± 15	120 ± 13	106 ± 16	103 ± 13	94 ± 15	91 ± 16
LP	109 ± 14	102 ± 14	103 ± 18	95 ± 22	90 ± 24	107 ± 23	106 ± 24
LV	4 ± 1	4 ± 1	4 ± 1	4 ± 1	5 ± 1	5 ± 1	4 ± 1
MV	6 ± 0	11 ± 1	9 ± 1	8 ± 1	8 ± 1	8 ± 1	8 ± 1
H	18 ± 1	19 ± 3	20 ± 0				20 ± 0
E	11 ± 5	12 ± 7	13 ± 5				16 ± 7
N	6 ± 2	3 ± 1	3 ± 1				3 ± 0
S	165 ± 97	56 ± 16	46 ± 13				67 ± 26

* All values represent the mean ± SEM obtained from 5 dogs at the specified time.

Thirty minutes after the termination of the histamine infusion when the animal had stabilized, endotoxin was injected, and the same hemodynamic or blood chemical measurements were obtained over the next 30 minutes.

Both the infusion of histamine systemically (group H-1) and the later injection of endotoxin induced an abrupt, sustained fall in SP, a transient rise in MP, a sustained fall in LP, and no change in LV. Infusion of histamine had no effect on MV, whereas endotoxin induced a typical transient portal hypertension. Infusion of histamine caused a rise in plasma E and a lesser rise in plasma H but failed to depress plasma S. Endotoxin induced a decline in S but no change in H. By the time endotoxin was administered, SP

TABLE V

*Effects of histamine injected into both systemic and portal venous circuits and of endotoxin on hemodynamic and neurohumoral parameters (group H-2)**

	Time, *minutes*						
	−5	+5	+10	+15	+20	+25	+30
	Systemic and portal infusion of histamine						
SP	125 ± 7	100 ± 8	76 ± 10	95 ± 8	92 ± 7	86 ± 6	91 ± 9
MP	114 ± 13	144 ± 20	169 ± 20	160 ± 28	154 ± 28	141 ± 27	132 ± 20
LP	134 ± 13	120 ± 9	109 ± 11	119 ± 14	129 ± 12	117 ± 12	120 ± 13
LV	3 ± 1	3 ± 1	2 ± 1	3 ± 1	2 ± 1	2 ± 0	3 ± 1
MV	6 ± 1	10 ± 1	14 ± 1	8 ± 7	7 ± 1	6 ± 1	6 ± 1
	Injection of endotoxin						
SP	92 ± 15	69 ± 12	64 ± 14	63 ± 14	63 ± 14	60 ± 15	75 ± 16
MP	154 ± 25	171 ± 25	181 ± 24	175 ± 26	165 ± 20	184 ± 34	153 ± 22
LP	130 ± 13	127 ± 13	120 ± 15	118 ± 14	121 ± 15	118 ± 15	123 ± 19
LV	4 ± 0	4 ± 0	4 ± 1	4 ± 0	4 ± 0	4 ± 0	4 ± 0
MV	9 ± 1	15 ± 2	13 ± 2	12 ± 2	11 ± 2	11 ± 2	9 ± 1

* Pressures represent the mean ± SEM obtained from 5 animals at the specified time.

TABLE VI

*Effects of acetylcholine and endotoxin on hemodynamic and neurohumoral parameters (group A-1)**

	Time, *minutes*						
	−5	+5	+10	+15	+20	+25	+30
	Systemic infusion of acetylcholine						
SP	136 ± 13	70 ± 23	77 ± 12	79 ± 10	75 ± 9	94 ± 13	102 ± 13
MP	120 ± 16	105 ± 13	116 ± 8	119 ± 7	123 ± 10	123 ± 12	122 ± 11
LP	132 ± 10	104 ± 12	117 ± 10	122 ± 15	122 ± 12	121 ± 9	135 ± 10
LV	4 ± 0	4 ± 0	5 ± 2	4 ± 0	4 ± 1	6 ± 2	6 ± 2
MV	9 ± 2	10 ± 2	10 ± 2	10 ± 2	10 ± 2	9 ± 1	9 ± 1
H	24 ± 8	24 ± 9	22 ± 7				21 ± 6
E	2 ± 0	4 ± 1	5 ± 1				4 ± 1
N	3 ± 1	3 ± 1	3 ± 1				3 ± 1
S	194 ± 75	205 ± 108	220 ± 122				127 ± 42
	Injection of endotoxin						
SP	103 ± 15	93 ± 6	82 ± 8	78 ± 9	71 ± 11	67 ± 12	63 ± 13
MP	113 ± 12	124 ± 13	160 ± 15	154 ± 16	148 ± 14	142 ± 12	135 ± 13
LP	127 ± 9	131 ± 8	128 ± 8	123 ± 16	117 ± 17	113 ± 16	112 ± 19
LV	4 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0
MV	9 ± 2	12 ± 0	14 ± 3	11 ± 1	10 ± 1	9 ± 1	9 ± 2
H	17 ± 4	29 ± 13	19 ± 7				19 ± 6
E	4 ± 1	9 ± 5	14 ± 5				18 ± 6
N	4 ± 2	5 ± 3	5 ± 2				6 ± 2
S	128 ± 34	64 ± 4	56 ± 11				71 ± 16

* All values represent the mean ± SEM obtained from 3 animals at the specified time.

was low and plasma E and N were elevated. These results are shown in Table IV.

Simultaneous infusion of histamine into femoral and mesenteric veins (group H-2) altered all hemodynamic parameters to resemble responses elicited by endotoxin, namely, a sustained fall in SP and LP, a sustained increase in MP, a transient increase in MV, and no change in LV (Table V).

2) *Acetylcholine.* Acetylcholine [7] was infused into either the jugular vein (group A-1) or into both the jugular and mesenteric veins (group A-2) of 8 dogs. The systemic dose varied from 250 to 2,500 μg base per minute for up to 30 minutes. The dose delivered into the portal circulation varied from 500 to 2,500 μg per minute for as long as 15 minutes. The various pressures and chemical determinations were obtained during acetylcholine infusion and during endotoxin shock at intervals similar to those in the histamine infusion studies (groups H-1 and H-2). Between the end of the acetylcholine infusion and the injection of endotoxin, a 30-minute interval was allowed for stabilization of the animal.

[7] Acetylcholine hydrochloride, Merck and Co., Inc., West Point, Pa.

Systemic infusion of acetylcholine (group A-1) and injection of endotoxin each caused a sustained fall in SP, a transient fall in LP, and no change in LV; increases in MP and MV were observed only after endotoxin. Both agents induced an increase in plasma E but no essential change in N. One of 3 dogs exhibited a marked rise in plasma H at 5 minutes after endotoxin that returned to pre-endotoxin values 5 minutes later. Only endotoxin induced a decrease in S values. These results are shown in Table VI.

Simultaneous infusion of acetylcholine into both mesenteric and femoral veins (group A-2) or the injection of endotoxin into a femoral vein induced the following common hemodynamic events: a sustained decline in SP and LP, a transient increase in MV, and no change in LV (Table VII). The increase in MP was observed only after endotoxin injection.

3) *Catecholamines.* In 6 dogs the hemodynamic responses to both catecholamine infusions and endotoxin were compared in the same dogs. SP, MP, LP, LV, and MV were monitored at 5-minute intervals during successive 30-minute periods of catecholamine infusion, during control, and after the injection of endotoxin. In 3 of

TABLE VII

*Effects of acetylcholine injected into both systemic and portal venous circuits and of endotoxin on hemodynamic and neurohumoral parameters (group A-2)**

	Time, *minutes*						
	−5	+5	+10	+15	+20	+25	+30
	Systemic and portal infusion of acetylcholine						
SP	130 ± 8	87 ± 20	85 ± 13	76 ± 12	60 ± 4	69 ± 5	76 ± 4
MP	162 ± 27	154 ± 21	142 ± 12	158 ± 21	148 ± 20	155 ± 26	169 ± 28
LP	142 ± 10	127 ± 19	127 ± 12	120 ± 9	123 ± 12	123 ± 10	125 ± 10
LV	3 ± 1	3 ± 1	3 ± 1	3 ± 1	2 ± 1	2 ± 1	3 ± 1
MV	7 ± 1	13 ± 2	10 ± 2	7 ± 1	6 ± 1	5 ± 1	5 ± 1
	Injection of endotoxin						
SP	103 ± 10	54 ± 17	58 ± 18	56 ± 18	54 ± 17	55 ± 19	55 ± 19
MP	155 ± 20	176 ± 17	182 ± 37	175 ± 43	180 ± 44	197 ± 43	200 ± 44
LP	128 ± 17	115 ± 19	118 ± 24	120 ± 23	122 ± 25	117 ± 21	125 ± 26
LV	5 ± 2	6 ± 1	6 ± 2	5 ± 1	6 ± 2	7 ± 3	6 ± 2
MV	6 ± 1	21 ± 7	13 ± 3	12 ± 4	11 ± 3	10 ± 2	9 ± 2

* Pressures represent the mean ± SEM obtained from 5 dogs at the specified time.

these dogs epinephrine was infused into a jugular vein (10 to 25 μg base per minute for 20 minutes). Samples were obtained for the determination of plasma H, E, N, and S at several times before and after infusing epinephrine and before and after injecting endotoxin. In the other 3 animals epinephrine was infused into a jugular vein, and norepinephrine [8] was infused simultaneously into a mesenteric vein (10 to 25 μg base per minute for 5 to 10 minutes). The finding in the control dogs (group PV) that portal venous norepinephrine concentrations rise more markedly than levels of this agent in the systemic circulation prompted the use of both catecholamines in this comparison of hemodynamic responses with changes induced by endotoxin.

[8] Levophed bitartrate, Winthrop Laboratories, New York, N. Y.

Systemic infusion of epinephrine (group EN-1) or the subsequent injection of endotoxin induced

TABLE VIII

*Effects of epinephrine and endotoxin on hemodynamic and neurohumoral parameters (group EN-1)**

	Time, *minutes*						
	−5	+5	+10	+15	+20	+25	+30
	Systemic infusion of epinephrine						
SP	147 ± 5	158 ± 2	152 ± 4	143 ± 12	139 ± 7	134 ± 7	127 ± 6
MP	137 ± 7	150 ± 12	126 ± 4	127 ± 6	129 ± 4	135 ± 6	133 ± 2
LP	157 ± 12	116 ± 26	145 ± 18	138 ± 12	142 ± 16	149 ± 7	156 ± 10
LV	3 ± 0	4 ± 1	4 ± 1	3 ± 0	3 ± 1	3 ± 1	3 ± 1
MV	10 ± 1	14 ± 1	12 ± 1	11 ± 1	11 ± 0	9 ± 1	8 ± 1
H	17 ± 1	16 ± 1	15 ± 3				17 ± 2
E	0 ± 0	14 ± 2	12 ± 2				1 ± 0
N	1 ± 0	1 ± 0	0 ± 0				1 ± 0
S	114 ± 9	119 ± 9	144 ± 21				108 ± 4
	Injection of endotoxin						
SP	122 ± 4	29 ± 21	23 ± 4	44 ± 19	36 ± 9	34 ± 8	36 ± 9
MP	129 ± 5	140 ± 22	163 ± 44	172 ± 19	178 ± 3	172 ± 16	173 ± 15
LP	158 ± 10	126 ± 29	118 ± 32	137 ± 22	152 ± 21	137 ± 19	132 ± 24
LV	3 ± 1	2 ± 0	3 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1
MV	8 ± 1	23 ± 5	11 ± 2	14 ± 2	13 ± 1	11 ± 1	10 ± 1
H	15 ± 2	14 ± 2	14 ± 1				16 ± 2
E	1 ± 0	6 ± 3	8 ± 4				9 ± 2
N	1 ± 0	2 ± 0	2 ± 0				5 ± 3
S	105 ± 19	50 ± 6	52 ± 13				53 ± 8

* All values represent the mean ± SEM obtained from 3 animals at the specified time.

TABLE IX

*Effects of catecholamines injected into both systemic and portal venous circuits and of endotoxin on hemodynamic and neurohumoral parameters (group EN-2)**

	Time, *minutes*						
	−5	+5	+10	+15	+20	+25	+30
	Systemic and portal infusions of catecholamines						
SP	120 ± 13	157 ± 10	138 ± 4	120 ± 6	125 ± 8	110 ± 8	98 ± 4
MP	122 ± 19	157 ± 17	127 ± 11	120 ± 8	130 ± 9	115 ± 6	132 ± 15
LP	128 ± 13	167 ± 6	157 ± 7	145 ± 13	140 ± 13	133 ± 9	128 ± 3
LV	5 ± 2	6 ± 2	5 ± 2	6 ± 2	5 ± 2	4 ± 2	5 ± 3
MV	8 ± 2	15 ± 1	13 ± 1	8 ± 1	8 ± 1	9 ± 2	8 ± 2
	Injection of endotoxin						
SP	90 ± 6	47 ± 9	44 ± 10	44 ± 10	44 ± 12	39 ± 17	46 ± 14
MP	133 ± 8	168 ± 7	185 ± 8	183 ± 3	195 ± 10	196 ± 21	198 ± 19
LP	133 ± 7	62 ± 8	80 ± 8	91 ± 11	94 ± 14	97 ± 17	100 ± 16
LV	4 ± 3	4 ± 3	4 ± 3	4 ± 4	4 ± 4	6 ± 2	6 ± 2
MV	8 ± 2	10 ± 2	9 ± 2	9 ± 2	9 ± 2	11 ± 2	11 ± 2

* Pressures represent the mean ± SEM obtained from 3 dogs at the specified time.

a transient rise in MV, a sustained fall in LP, and no change in LV. Endotoxin induced a persistent rise in MP and an early sustained decline in SP. Both agents caused an increase in plasma E and no change in H. Epinephrine induced a fall in N and no change in S, whereas the injection of endotoxin was followed by a decline in S and no essential change in N concentrations. These results appear in Table VIII.

Simultaneous infusion of epinephrine systemically and norepinephrine into the portal circulation (group EN-2) or endotoxin resulted in transient portal hypertension and no change in LV (Table IX). The catecholamine infusions induced an abrupt rise in SP that fell late and transient increases in MP and LP. Endotoxin in these same dogs induced an abrupt sustained fall in SP and LP and a prolonged rise in MP.

4) Serotonin. In another 5 dogs serotonin [9] was infused into a jugular vein (125 to 250 μg base per minute for 16 to 31 minutes) starting 1 minute before the injection of endotoxin (group S). Since serotonin concentrations in plasma exhibit a marked decline in response to the injection of endotoxin, it appeared reasonable to infuse this agent in amounts calculated to prevent the fall in circulating levels during endotoxemia. In 3 of these dogs, plasma H, E, N, and S concentrations were measured before and at 5, 10, and 30 minutes after injecting endotoxin. Hemodynamic changes (SP, MP, LP, LV, and MV) were monitored at 5-minute intervals throughout the test period in all 5 dogs.

[9] 5-Hydroxytryptamine creatinine sulfate, Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE X

*Effects of injection of endotoxin during prolonged systemic infusion of serotonin (group S)**

	Time, *minutes*						
	−5	+5	+10	+15	+20	+25	+30
	Infusion of serotonin and injection of endotoxin						
SP	141 ± 5	75 ± 15	64 ± 15	54 ± 15	56 ± 12	59 ± 13	61 ± 14
MP	151 ± 12	226 ± 34	229 ± 30	202 ± 29	194 ± 29	186 ± 22	185 ± 20
LP	164 ± 16	129 ± 27	140 ± 35	96 ± 25	109 ± 33	102 ± 27	106 ± 25
LV	3 ± 1	4 ± 1	3 ± 1	2 ± 0	3 ± 1	3 ± 1	4 ± 1
MV	8 ± 1	23 ± 4	15 ± 2	10 ± 1	10 ± 1	9 ± 1	9 ± 1
H	15 ± 2	16 ± 2	17 ± 3				18 ± 0
E	2 ± 0	10 ± 6	8 ± 2				7 ± 2
N	1 ± 0	1 ± 0	1 ± 0				1 ± 0
S	95 ± 18	99 ± 35	163 ± 55				120 ± 12

* All values represent the mean ± SEM obtained from 3 or 5 animals at the specified time.

TABLE XI

*Responses of systemic arterial pressure and portal venous pressure to the injection of histamine, epinephrine, norepinephrine, and serotonin under control conditions and during endotoxemia**

Group		Time, *minutes* −15	−10	−5	+5	+10	+20	+30
		Systemic arterial pressures						
SH	SP	152 ± 7	150 ± 8	150 ± 9	132 ± 3	133 ± 4	137 ± 4	137 ± 7
	D	−73 ± 2	−67 ± 3	−68 ± 3	−55 ± 0	−62 ± 2	−67 ± 2	−72 ± 4
SE	SP	138 ± 11	142 ± 9	150 ± 6	98 ± 15	122 ± 14	130 ± 10	145 ± 10
	D	+27 ± 2	+30 ± 3	+27 ± 3	+50 ± 10	+28 ± 3	+27 ± 4	+15 ± 5
SN	SP	118 ± 2	120 ± 3	127 ± 9	75 ± 23	90 ± 16	110 ± 12	117 ± 8
	D	+73 ± 9	+72 ± 10	+68 ± 11	+35 + 10	+45 ± 9	+43 ± 8	+45 ± 10
SS	SP	132 ± 6	127 ± 10	145 ± 13	88 ± 13	135 ± 12	132 ± 9	137 ± 10
	D	+32 ± 4	+42 ± 4	+38 ± 7	+65 ± 8	+37 ± 2	+47 ± 4	+38 ± 3
		Portal venous pressures						
PH	MV	9 ± 1	9 ± 1	9 ± 1	22 ± 4	16 ± 2	13 ± 1	11 ± 1
	D	+4 ± 1	+4 ± 0	+5 ± 0	0 ± 1	+1 ± 1	+2 ± 1	+4 ± 2
PE	MV	7 ± 2	7 ± 2	7 ± 2	13 ± 4	10 ± 2	8 ± 2	7 ± 2
	D	+4 ± 1	+4 + 1	+3 ± 1	0 ± 0	+3 ± 1	+3 ± 2	+3 ± 1
PN	MV	8 ± 1	8 ± 1	8 ± 1	21 ± 6	17 ± 5	10 ± 2	10 ± 2
	D	+4 ± 0	+4 ± 1	+4 ± 0	+3 ± 3	−1 ± 1	0 ± 0	+1 ± 1
PS	MV	8 ± 1	7 ± 1	7 + 1	17 ± 4	10 ± 1	7 ± 0	5 ± 1
	D	+3 ± 1	+4 ± 1	+3 ± 1	+3 ± 3	+3 ± 2	+3 ± 2	+4 ± 1

* Abbreviations: SP = mean systemic arterial pressure at the time of injection; D = maximal change in pressure after injection; MV = mean mesenteric venous pressure at the time of injection; SH, SE, SN, and SS = groups in which either histamine, epinephrine, norepinephrine, or serotonin was injected into a femoral vein; PH, PE, PN, and PS = groups in which either histamine, epinephrine, norepinephrine, or serotonin was injected into a mesenteric vein. All values represent the mean ± SEM obtained from 3 dogs at the specified time.

These animals exhibited changes typical of endotoxin alone, despite the prevention of a decline in S concentrations (Table X). Thus SP and LP decreased, MP and MV increased, and LV was unchanged; plasma E increased, and N and H were unchanged.

d) Sensitivity experiments

The animals in these experiments were maintained on a respirator, and heparin was not administered. In 12 dogs pressures were monitored in the left femoral artery. These dogs were divided into 4 equal groups according to the agent injected into a femoral vein: histamine, epinephrine, norepinephrine, or serotonin. After 3 control responses to a dose of the agent fixed for each animal to elevate or depress mean pressure approximately 40%, endotoxin was injected. The doses used in μg per kg body weight were these: histamine, 5; epinephrine, 2; norepinephrine, 2; and serotonin, 25. The same dose of the agent was repeated at 5, 10, 20, and 30 minutes after endotoxin, and the responses were compared with control responses to see whether endotoxemia enhanced or attenuated the vascular changes induced by these neurohumoral agents.

In 12 other dogs a mesenteric vein was exposed through a left subcostal laporatomy, and pressures were monitored in the vein before and after injecting endotoxin. Again groups of 3 dogs each received one of the 4 neurohumoral agents in doses fixed for each animal to elevate portal pressure approximately 40%. Histamine (0.5 μg per kg), epinephrine (0.6), norepinephrine (1.0), or serotonin (33) was injected into another mesenteric vein. Mean pressures were obtained according to the time schedule outlined above.

The systemic arterial depressor response to histamine was not increased at any observed time after endotoxin. The absolute pressure change induced by histamine was minimal at 5 minutes after endotoxin. Similarly the portal pressor response to histamine was not increased after the injection of endotoxin. The periods of least response to histamine occurred when portal and systemic pressures were maximally affected by endotoxin. The results from all sensitivity experiments appear in Table XI.

The systemic arterial response to epinephrine was enhanced 5 minutes after endotoxin and later returned to preinjection responsiveness; the portal

pressor response was diminished at 5 minutes. The systemic and portal pressor responses to norepinephrine were reduced from control responses at all observation periods after injection of endotoxin. Systemic arterial pressor responses to serotonin were increased from control after endotoxin, but portal pressor responses were unaltered.

Discussion

The present study was designed to examine the possible role of histamine, epinephrine, norepinephrine, and serotonin in the pathogenesis of the early vascular events of endotoxin shock. The experimental rationale was based on four requirements that we believe should be fulfilled to consider any agent as the triggering device of endotoxin shock. These criteria are: *1*) either plasma levels of the agent or vascular responsiveness to the agent should increase shortly after the administration of endotoxin; *2*) the major early vasomotor and chemical events of endotoxin shock should be reproduced by infusion of the agent, and these changes should be unique for that agent; *3*) pharmacological substances that "deplete" the tissues or blood of the agent should alter the early vascular and chemical events of endotoxin shock; and *4*) specific end organ antagonists should reduce the vascular responsiveness of the animal to endotoxin.

If these four requirements are valid, then apparently neither histamine, epinephrine, norepinephrine, nor serotonin qualifies as the "trigger mechanism" of endotoxin shock. None of these proposed intermediaries was unique among naturally occurring neurohumoral substances in its ability to mimic the major early vascular responses to endotoxin, and prior treatment of dogs with drugs that reduce tissue stores of these vasoactive substances failed to prevent the typical hemodynamic events of endotoxemia. The initial hypotension induced by endotoxin can scarcely be attributed to the increased concentrations and vascular responsiveness to epinephrine when the over-all early effect of an infusion of this agent is to raise systemic arterial pressure. Similarly, the prevention of a decline in serotonin concentrations did not prevent endotoxin shock. Furthermore, in a previous report (16) we found that a variety of effective end organ antagonists (including antihistaminics, atropine, reserpine, dichloroisoproterenol, Nethalide,[10] and cyproheptadine) were unable to block the major vascular events of endotoxin shock.

The nature of this study imposes certain limitations to the interpretation of our results. Obvious technical shortcomings include the use of the surgically traumatized, anesthetized dog and his pump-traumatized circulation as the hemodynamic model during endotoxemia and toxic drug infusions. Certain canine vascular responses to endotoxin are unique to that species (26), although this experimental syndrome is remarkably reproducible (18). Regional perfusion and measurement of large vessel pressure changes yield only net changes in resistance across the organ, which may not correspond to events occurring in the microcirculation. Similarly, measurement of plasma concentrations of neurohumoral agents may be an unreliable index of turnover rates and of neurohumoral activity in the microcirculation. Furthermore, the comparison of vascular responsiveness to these neurohumoral substances during control conditions and endotoxin shock may not be valid because of the markedly different conditions of the circulation (changes in vascular tone and flow can alter pressure). The greatly constricted vascular bed of endotoxemia may be unresponsive to exogenous agents, and the change in blood flow may alter the amount of each agent that actually reaches the microcirculation. In addition, the drugs used to deplete tissue stores of histamine, catecholamines, and serotonin were not totally satisfactory, although studies reported elsewhere indicate that tissue levels of the neurohumoral substances are diminished by the drugs (23, 27–31).

Among the various systems implicated in the pathogenesis of endotoxin shock, histamine has received considerable attention in recent years. This has been primarily because plasma levels of histamine increase gradually after the administration of lethal amounts of endotoxin (10, 32) and because endotoxin enhances histidine decarboxylase activity (3). The findings that compound 48/80, a potent histamine-releasing com-

[10] 2-(*d*-Hydroxy-3-isopropyl aminoethyl naphthalene), Ayerst Laboratories, New York, N. Y.

pound, and histamine both induce vascular changes similar to those induced by endotoxin has prompted investigators to assign a primary role to histamine as the triggering device of endotoxin shock (4). We have also demonstrated a similarity between many vascular and chemical responses of the dog to endotoxin and histamine. In our studies, however, plasma levels of histamine did not increase with sublethal amounts of endotoxin, which induced the typical hemodynamic events of endotoxemia. In addition, vascular sensitivity to histamine was not increased during the first 30 minutes of endotoxemia. Furthermore, the similarities between the hemodynamic and chemical effects of histamine and endotoxin are not unique to histamine. Acetylcholine also simulated most of these chemical and vascular responses to either histamine or endotoxin. However, neither histamine nor acetylcholine induced the dramatic and invariable decrease in plasma serotonin levels observed in normal animals receiving endotoxin, whereas histamine infusion uniquely elevated plasma histamine concentrations. Acetylcholine has not been implicated in endotoxin shock, and the atropinized dog responds to endotoxin in a manner typical of control dogs (16). Compound 48/80 and cortisone, administered in doses that presumably diminished both mast cell (30, 31) and acetylated tissue histamine (23), failed to alter the vascular and chemical responses to endotoxin. Also, in a previous study we found that massive doses of antihistamines that blocked the depressor response to histamine did not alter the vascular effects of sublethal amounts of endotoxin (16). The possibility exists that endogenously produced histamine ("induced histamine") is the trigger mechanism in endotoxin shock and that this hypothetical material is unaffected by either antihistaminics or agents that diminish histamine in the tissues. Histidine decarboxylase activity is not, however, significantly elevated in the first hour after the administration of endotoxin (3). Furthermore, in shock induced by beta mercaptoethylamine, whose vascular events resemble those of endotoxin shock, histamine is released (33), and the vascular changes are blocked by antihistaminics (34).

Our findings that the plasma concentrations of histamine do not increase early in endotoxemia and that antihistaminics and compound 48/80 are ineffective in modifying the hemodynamic responses to endotoxin are at variance with the reports of others (1, 4, 10, 17). This may be attributable to the differences between the doses of endotoxin employed in their studies and the amounts of material we injected. In our experiments a sublethal (approximately LD_{50}) dose was used, whereas other workers have utilized a dose many times the lethal amount. Massive doses of endotoxin may precipitate an anaphylactoid reaction (1) in which there is a release of histamine and which responds to antihistaminics (17).

The catecholamines have been implicated in the genesis of endotoxin shock for the following reasons: *a*) plasma concentrations and vascular sensitivity are altered during the early phase of endotoxemia (5–7, 11, 13); *b*) adrenergic blocking agents protect against certain effects of endotoxin (6, 35–36); and *c*) both agents induce hepatic venoconstriction (1, 37), splanchnic pooling (36, 38), a diminished circulating blood volume (36, 39), vasoconstriction—splanchnic (1, 40), renal (41–43), and cutaneous (35, 40), dilation of the circulation in the heart (44, 45) and skeletal muscle (46), and an over-all reduction in total peripheral resistance (47, 48). In our studies, however, systemic arterial pressure did not fall abruptly during catecholamine infusions, pretreatment with reserpine did not prevent usual responses to endotoxin, and pharmacological antagonists to catecholamines did not abolish all the hemodynamic responses to endotoxin (16).

Serotonin has also been implicated in endotoxin shock (7–9, 49). In our experiments simultaneous infusion of serotonin during endotoxemia in amounts that prevented the decline in plasma serotonin levels did not alter the typical responses to endotoxin. Furthermore, dogs treated with agents that diminish tissue or blood serotonin exhibited vascular responses to endotoxin similar to control animals.

The foregoing discussion underscores the complexity of endotoxin shock. From our results neither histamine, epinephrine, norepinephrine, nor serotonin seems to qualify as the primary mediator or the essential "trigger substance" in endotoxin shock, if the four criteria previously outlined are accepted. On the basis of these experiments and the endotoxin literature, to assign primacy to any of the suggested vascular

mediators of endotoxin shock is speculative. In addition, other complex and seemingly unrelated systems appear to be deeply involved in endotoxemia, including the adrenal cortical steroids (50), vasoactive polypeptides (51), permeability factors (52), coagulation mechanisms (53, 54), carbohydrate metabolism (55–57), and various enzymatic processes (54, 58–59).

Summary

The role of histamine, catecholamines, and serotonin was investigated in the early phase of endotoxin shock in the dog. Histamine induced hemodynamic alterations similar to endotoxin; however, these changes could also be induced by larger amounts of acetylcholine. Plasma concentrations of histamine were not elevated by sublethal amounts of endotoxin, and vascular responsiveness to histamine was reduced during endotoxemia. Pretreatment of dogs with compound 48/80 failed to alter responses to endotoxin. Epinephrine concentrations increased, and arterial sensitivity to epinephrine was enhanced during endotoxemia. Pretreatment of animals with reserpine, however, did not alter the events of endotoxin shock, and infusions of catecholamines incompletely duplicated the responses to endotoxin. During endotoxemia plasma serotonin concentrations were reduced, and arterial sensitivity to serotonin was heightened. Replacement of serotonin during endotoxin shock failed to alter the events induced by endotoxin. On the basis of our experiments, apparently none of these agents can be considered as the primary mediator of the early phase of endotoxin shock.

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