Cerebrospinal Fluid Levels of Endotoxin During Endotoxemia (37444)

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Despite intensive research, the complete pathogenesis of septic shock due to gram-negative bacteria is still a perplexing problem (1). Endotoxemia, which may result from sepsis, is the prime subject of research in the effort to elucidate the pathophysiological mechanisms involved. It has been suggested that a direct action of endotoxin on the central nervous system (CNS) may be responsible for some of its effects (2–6). However, Weil *et al.* presented data showing that endotoxin did not act primarily via the CNS (7).

A prerequisite for a direct CNS effect of systemic endotoxin is that the molecule contacts CNS elements. Using the pyrogenic effect in rabbits as an assay, Bennett *et al.* presented evidence that endotoxin was found in the cerebrospinal fluid (CSF) of dogs within 15–30 min after intravenous injection of *Shigella* endotoxin (8). However, studies employing radioactively-labeled endotoxins have not found evidence of endotoxin in the brain parenchyma (9–11).

The purpose of this investigation was to determine if intravenously injected endotoxin could gain access to the CSF and thus have possible direct CNS effects. The *Limulus in vitro* endotoxin assay was utilized to detect endotoxin in CSF as well as document blood levels during the period of the experiment.

Methods and Materials. Eighteen adult mongrel dogs of either sex weighing 8–22 kg were anesthetized with an intravenous injection of urethane (640 mg/kg) and chloralose (54 mg/kg). Arterial pressure was measured by passing a catheter through the femoral artery to the thoracic aorta. This was connected to a Statham P23db pressure transducer and recorded on an Electronics for Medicine oscilloscopic recorder. Arterial blood gasses and pH were measured with the Instrumentation Laboratory pH/Gas Analyzer (Model 113) using 3.5 ml of blood taken from the arterial catheter. Microhematocrit measurements were also taken from these samples. A second catheter was passed through the femoral vein to the level of the inferior vena cava for injecting endotoxin and for withdrawing blood used for the plasma endotoxin determinations. CSF samples were collected in pyrogen-free plastic containers from an 18-gauge spinal needle placed in the cisterna magna by an atlanto-occipital puncture. Venous blood was collected in sterile syringes and put into B-D vacutainers containing sodium heparin.

After taking control measurements of arterial pressure, blood gasses, and hematocrit and collecting control samples of CSF and venous blood, endotoxin dissolved in 50 ml of normal saline was administered intravenously as a single bolus. Six dogs were given a low dose (0.5 mg/kg) of purified *E. coli* endotoxin (055:B5 Lipopolysaccharide W, Difco Laboratories, Detroit, Mi.) and twelve dogs were given a high dose (5 mg/kg). The measurements and samples were repeated at 5 min, 30 min, 1 hr, and 2 hr after endotoxin.

CSF and plasma levels of endotoxin were measured by the *Limulus in vitro* endotoxin assay as described by Levin *et al.* and Reinhold and Fine (12, 13). This assay is the most sensitive test for gram-negative bacterial endotoxin currently available. CSF was checked for blood contamination by determining the red cell count in each sample with a hemocytometer. This was a necessary pre-

caution in order to determine whether endotoxin found in the CSF was the result of a traumatic tap. Of the twelve high-dose dogs, six had positive CSF endotoxin assays and were considered as a separate group for the purpose of discussion. The three groups of six dogs were designated as low-dose, negative high-dose, and positive high-dose groups. A comparison of variables between the two high-dose groups at control and at the various time intervals following endotoxin was made with an unpaired t test. A p value of less than 0.05 was considered to be statistically significant.

Results. Mean arterial pressure, hematocrit,

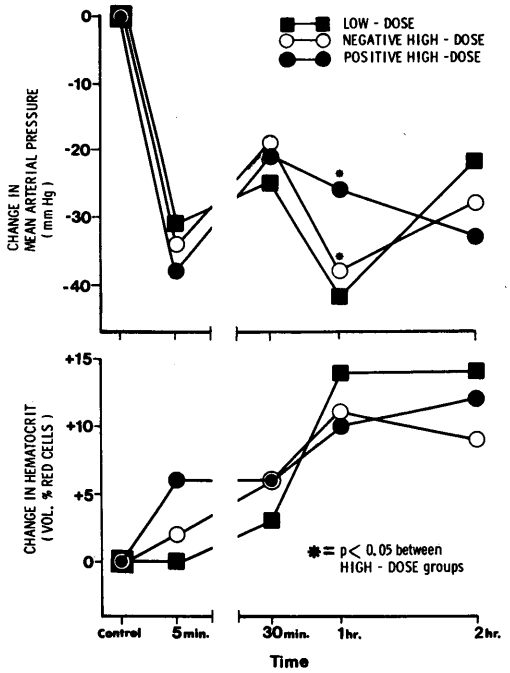

FIG. 1. Changes in mean arterial pressure and hematocrit from control values following iv injection of *E. coli* endotoxin, 0.5 mg/kg (low dose) or 5 mg/kg (high dose). Positive or negative refers to the endotoxin assay of the cerebral spinal fluid. The CSF samples of all low-dose dogs were negative for endotoxin. Each group consisted of six dogs. There were no significant differences between high-dose groups, except as indicated (*). The control values (± SE) of the low-dose, negative high-dose, and positive high-dose groups, respectively were: 122 ± 5, 106 ± 7, and 125 ± 6 mm Hg for mean blood pressure; and 39 ± 2, 35 ± 3, and 40 ± 1% for hematocrit.

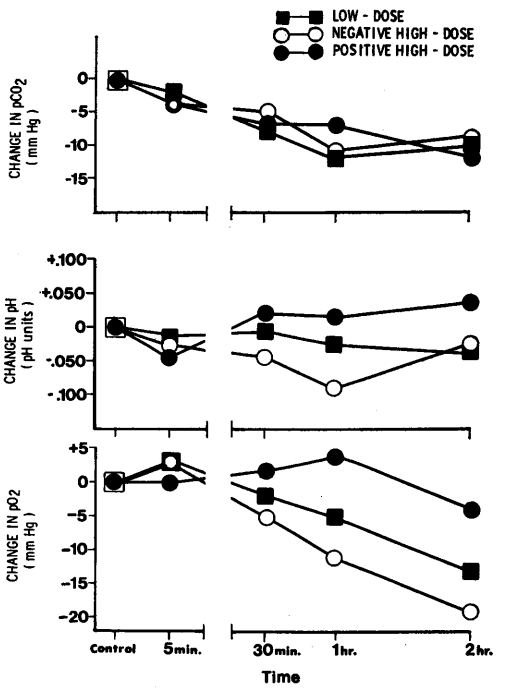

FIG. 2. Changes in blood gasses and pH in the three groups of dogs following iv injection of *E. coli* endotoxin. See Fig. 1 for explanation. The control values (± SE) of the low-dose, negative high-dose, and positive high-dose groups, respectively, were: 35 ± 1, 35 ± 1, and 39 ± 2 mm Hg for pCO_2; 7.349 ± 0.015, 7.393 ± 0.007, and 7.339 ± 0.013 pH units for pH; and 72 ± 6, 86 ± 5, and 78 ± 6 mm Hg for pO_2.

blood gasses and pH. The changes in mean arterial pressure and hematocrit from control values after injection of endotoxin are shown in Fig. 1. The changes in the blood gasses and pH are shown in Fig. 2. The three groups had similar responses and, in general, were typical of those of dogs in endotoxin shock as reported by other investigators (14–16). There were no significant differences between the two high-dose groups except at 1 hr, in which case the mean arterial pressure of the negative high-dose group dropped more than that of the positive high-dose group.

Plasma levels of endotoxin. Table I shows the mean plasma levels of endotoxin in micrograms per milliliter (μg/ml). None of the control samples demonstrated a positive endotoxin reaction. At 5 min after endotoxin administration the low-dose group had a mean

TABLE I. Mean Plasma Levels of Endotoxin[a] (μg/ml) $\pm$ SE.

Group[b]	Control	5 min	30 min	1 hr	2 hr
Low-dose	0	38 $\pm$ 6	8 $\pm$ 1	3 $\pm$ 1	0.8 $\pm$ 0.1
Negative[c] High-dose	0	417 $\pm$ 15	105 $\pm$ 19	15 $\pm$ 2	6 $\pm$ 2
Positive[c] High-dose	0	583 $\pm$ 80	200 $\pm$ 69	55 $\pm$ 18	14 $\pm$ 5

[a] *E. coli* endotoxin was given iv, 0.5 mg/kg (low-dose) or 5 mg/kg (high-dose) after control venous blood samples were taken.

[b] Each group consisted of six dogs.

[c] Negative or positive refers to the endotoxin assays of the CSF.

plasma endotoxin concentration of 38 μg/ml, which decreased to 0.8 μg/ml at the end of 2 hr. In the negative high-dose group, at 5 min the mean plasma level of endotoxin was 417 μg/ml and decreased to 6 μg/ml at 2 hr. In the positive high-dose group, the 5-min and 2-hr mean plasma endotoxin concentrations were 583 and 14 μg/ml, respectively. There were no statistically significant differences in the plasma levels of endotoxin between the two high-dose groups.

Cerebrospinal fluid levels of endotoxin. Table II shows the CSF levels of endotoxin in nanograms per milliliter (ng/ml) and indicates the amount of hemorrhage into the CSF as evidenced by the RBC's/mm^3 for each sample. None of the control samples in the three groups had positive endotoxin reactions. In the low-dose group all the CSF samples were negative for endotoxin despite two of the six dogs having apparent traumatic cisternal taps.

Of the dogs that received the high doses of endotoxin, only those that had an appreciable amount of red cells in the CSF had significant amounts of CSF endotoxin. An appreciable amount of hemorrhage was considered to be a RBC-count of 20/mm^3 or more in the samples taken after control. The one exception was dog no. 14 which had 100 ng/ml of endotoxin at 1 hr and 2 hr but only 4 RBC's/mm^3. Dogs numbers 13 and 17 of the negative high-dose group each had 1 ng/ml of endotoxin in the 1-hr and 2-hr samples. In all the other dogs studied no endotoxin could be detected in the CSF.

Discussion. Septic shock occours most frequently in hospitalized patients with underlying debilitating diseases. Predisposing factors include leukemia, malignancy, cirrhosis, severe burns, infant prematurity, traumatic injury, and hemorrhage (17). Sepsis, under these conditions, has a poor prognosis and results in high mortality rates. Rational treatment will require a better basic understanding of the initiating mechanisms. Since it has been proposed that endotoxin may have a direct action on the CNS, this study was undertaken to ascertain whether such a large molecule could gain access to the cerebrospinal fluid (CSF) from the systemic circulation.

A necessary requirement for a substance to have direct action on the CNS is that it comes into direct contact with the neural elements of the brain or spinal cord. In this study it was assumed that the presence or absence of endotoxin in the CSF would indicate whether or not endotoxin could have direct CNS action. In making this assumption, passage of molecules across two interfaces must be considered: (*1*) across the cerebral capillary endothelium into the brain parenchyma (blood–brain barrier), and (*2*) across the endothelium of the choroid plexuses or arachnoid membrane into the CSF (blood–cerebrospinal fluid barrier). If a molecule is able to cross the blood–brain barrier into the brain parenchyma, would it diffuse from the brain into the CSF? And if a molecule is able to cross the blood–cerebrospinal fluid barrier, would it diffuse from the CSF into the brain? Results from both rate diffusion experiments (18) and from ultramorphologi-

TABLE II. Cerebrospinal Fluid Levels of Endotoxin and Erythrocytes

GROUP	Dog No.	Endotoxin (ng/ml)					RBC's / mm^3				
		Control	5 min	30 min	1 hr	2 hr	Control	5 min	30 min	1 hr	2 hr
Low Dose	6	-	0	0	0	0	-	-	-	-	-
Endotoxin	9	0	0	0	0	0	5	51	60	19	14
(.5 mg/kg)	19	0	0	0	0	-	1	1	0	4	-
	20	0	0	0	0	0	0	0	0	0	0
	21	0	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	7	3	2	15	393
	Mean	0	0	0	0	0	3	11	12	8	81
High Dose	10	0	0	0	0	0	1	0	1	0	1
Endotoxin	12	0	0	0	0	0	2	0	0	1	1
(5 mg/ml)	13	0	0	0	1	1	0	0	0	0	0
	17	0	0	0	1	1	39	1	0	2	1
	22	0	0	0	0	0	2	2	3	7	17
	23	0	0	0	0	0	0	1	1	0	1
	Mean	0	0	0	0	0	7	1	1	2	3
High Dose	3	0	20	-	75	20	36	17,000	-	17,000	36,000
Endotoxin	8	-	0	100	100	50	-	38	377	377	677
(5 mg/kg)	11	0	100	100	10	10	414	346	1,275	6,000	20,000
	14	0	0	1	100	100	3	1	0	4	4
	15	0	2	50	100	100	27	92	55	115	272
	18	0	0	0	5	5	2	1	1	123	95
	Mean	0	20	50	65	48	96	2,913	342	3,937	9,508

cal studies (19) have indicated this to be true in both cases. Therefore, it follows that if a substance is absent from the CSF, it would also be absent from the brain parenchyma, providing sufficient time were allowed for diffusion to take place.

Using a ventriculocisternal perfusion preparation in dogs, the volume contained in the lateral, third and fourth ventricles, the subarachnoid space of the posterior fossa, and the cisterna magna was estimated at 3.0–7.6 ml, or an average of 4.9 ml (20). The rate of CSF formation from these locations was found to be 0.047 ml/min (20). Complete turnover of the CSF within these compartments would thus occur about every 1.7 hr. Therefore, the two-hour duration of the present experiments was felt to be sufficient time for potential CSF endotoxin to appear.

CSF was drawn from the subarachnoid space at the level of the cisterna magna for two reasons: (*1*) sampling from this site is convenient and produces minimal trauma, and (*2*) the CSF from this location is a mixture of fluid formed both in the ventricles and in the subarachnoid space (20).

In this study the results indicated that only in the instances when cerebral blood vessels were damaged in preparation was endotoxin detected in the CSF. In the positive high-dose

group all the CSF samples had relatively large numbers of erythrocytes. The one exception, dog no. 14, had a small number of RBC's in the CSF, but also had the highest plasma levels of endotoxin of all the dogs studied. The bleeding into the CSF was caused by an inaccurately placed spinal needle at the time of the atlanto-occipital puncture. The CSF endotoxin in this group was probably the result of leakage from capillaries that were disrupted by the needle and therefore the results from these dogs were considered invalid.

In the low-dose group no endotoxin was found in the CSF at any time. Two dogs in this group showed a small amount of cerebral bleeding but still had negative CSF endotoxin, probably because the relatively low plasma levels of endotoxin in conjunction with the low-level bleeding was not sufficient to contaminate the CSF. The dogs in the negative high-dose group received ten times the amount of endotoxin as the low-dose dogs and yet the CSF samples had negative endotoxin assays. There was also a minimum amount of bleeding in this group. Two of the dogs in the negative high-dose group showed a minute amount of CSF endotoxin.

The plasma clearance of endotoxin has been previously characterized in the dog (11), and in other species (13, 21, 22). Our results are in general agreement with the rapid decrease of plasma endotoxin concentration during the first two hours. During this period when the plasma levels of endotoxin were at the highest, no endotoxin appeared in the CSF in spite of the enormous concentration gradient of endotoxin that existed between the CSF and blood. In view of these findings, it would appear that endotoxin does not cross the blood–brain barrier. This is not surprising considering it has a molecular weight of one million or greater (23).

Previous studies utilizing radioactively labeled endotoxin have found no evidence of endotoxin in the brain tissue after intravenous administration (9–11). In at least one of the papers it was reported that the blood was carefully washed from the tissues to be examined before the radioactivity was measured (11). Therefore, no contamination from the blood would be expected. The results of Bennett *et al.* (8), which showed that endotoxin was detected in the CSF of dogs 15–30 min after intravenous administration, might be explained by contamination from cerebral hemorrhage. These investigators placed a needle in the cisterna magna for sampling CSF, but did not report whether evidence of bleeding was present or not. We have found that the amount of bleeding that could cause leakage of endotoxin into the CSF is not always apparent by gross inspection.

Several investigations have shown that some of the effects of systemic endotoxin can be reproduced by injecting endotoxin directly into the CSF (3–6). Except in the cases where the source is a cerebral abcess, these results could not be extrapolated to the endotoxin shock syndrome if the lipopolysaccharide does not normally reach the brain. The findings of this study show that in dogs, systemic endotoxin does not reach the CSF within the first two hours after intravenous injection and suggest that the early manifestations of endotoxin shock are not mediated by a direct effect of endotoxin on the central nervous system. However, these results do not exclude the possibility that the CNS is directly acted upon by a substance which might be activated or released into the blood by endotoxin or that endotoxin may penetrate into the CSF after a longer time interval.

Summary. Dogs were given *E. coli* endotoxin intravenously to produce the typical hemodynamic and respiratory shock state. Plasma and cerebrospinal fluid levels of endotoxin were measured by the *Limulus in vitro* endotoxin assay at predetermined intervals for two hours. During the period when the plasma concentration was at its highest, no endotoxin was detected in the cerebrospinal fluid. These results suggest that systemic endotoxin does not produce its effects by acting directly on the central nervous system during the early phase of endotoxemia.

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