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Intracerebroventricular Administration of Leukocytic Endogenous Mediators (LEM) in the Rat[1] (39560)

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The systemic injection of rabbit leukocyte-derived factors into rats has been shown to depress plasma iron (1) and zinc (2) values, increase plasma copper concentrations (3), enhance flux of amino acids to the liver (4, 5), and increase synthesis of acute-phase globulins (4). Kampschmidt *et al.* (6) intracisternally injected rabbit LEM into rats and observed a decrease in plasma iron concentration and an increase in blood neutrophils. Intracisternal injections of LEM into rabbits produced a marked fever at much lower doses than if given systemically, suggesting a primary site of action in the central nervous system (7). Depending upon the procedure of isolation, leukocyte-derived factors have been referred to as endogenous pyrogen, leukocyte pyrogen, or leukocytic endogenous mediator. Because of the multiplicity of activities associated with this crude preparation, the term "leukocyte endogenous mediator" (LEM) was assigned to these unpurified substances.

Rabbit LEM was tested in rat 2B comparable with the majority of the work being done in this area, because Kampschmidt and Upchurch were unable to show a leukocytic pyrogen from rat polymorphonuclear leukocytes (8). LEM obtained from glycogen-induced peritoneal exudates in rabbits was injected into the lateral cerebral ventricle of rats in the present study to determine if alterations in plasma trace metal concentration, amino acid flux, and acute-phase globulin synthesis could be produced by this route.

Materials and methods. LEM (1×10^8 PMN cells/ml) was prepared from rabbit polymorphonuclear (PMN) leukocytes obtained from glycogen-induced peritoneal exudates in the standard fashion (4). A portion of the LEM preparation was inactivated in a boiling water bath for 30 min. Following the boiling period, the crude material was removed by centrifugation (Beckman microfuge for 5 min) and the supernatant represented the heat-treated LEM (ΔLEM).

Male Fisher-Dunning rats with initial body weight of 200 g were used in this experiment. The rats were kept in individual cages with the ambient temperature of 21 ± 0.5°. Under sodium pentobarbital anesthesia (40 mg/kg, ip), a guide cannula (Plastic Products Co., Roanoke, Va.) was implanted aseptically into the right lateral cerebral ventricle. The stereotaxic coordinates (9) were 0 mm from the bregma, 1.5-mm lateral to the sagittal suture, and 4.5 mm in depth from the surface of the skull. The rats were given 12 days to recover from the operation before an injection was made according to the procedure of Feldberg and Saxena (10). Another group of rats were injected ip with an equivalent volume of LEM.

Rectal temperature was recorded with a thermistor probe (Yellow Spring Instrument Co., Yellow Springs, Ohio), inserted 6.5 cm into the rectum every 30 min. After the temperature was recorded, animals were placed back in their cages and allowed to move freely.

At various time intervals after the intracerebroventricular (icv) administration of the various substances, the rats were anesthetized with halothane and approximately 1 ml of blood was collected by orbital bleed-

[1] In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

ing. A differential and white blood cell count was determined from the heparinized blood sample. The blood was then centrifuged at 3000 *g* for 10 min in a refrigerated centrifuge. Plasma zinc, iron, and copper concentrations were determined by atomic absorption spectrophotometry (11). α-2-Macrofetoprotein was analyzed by radial immunodiffusion (12). Some of these rats were pretreated with [1-^{14}C]α-aminoisobutyric acid ([^{14}C]AIB) so that amino acid flux to the liver could be determined according to the method of Wannemacher *et al.* (13). Statistical analyses were done using Student's *t* test for paired or unpaired variates.

Results. Figure 1 illustrates the hyperthermic response to icv LEM (20 μl/rat) in 20 different rats. Temperature began to rise (2–3°) steeply within 60 or 90 min of each injection. The hyperthermic response was relatively long-lasting but did not exceed 24 hr. Heat-treated icv LEM (20 μl/rat) did not alter normal rectal temperatures. Heated endotoxin (Δendotoxin; boiling in water bath for 30 min) and endotoxin (10 ng/20 μl/rat) both produced an icv hyperthermic response (1–2°) which began to rise 90 or 120 min after the injection. The endotoxin hyperthermic response appeared to have the same duration as that caused by LEM. After injection of 10 or 50 μl of icv LEM, the hyperthermic response was similar to that after 20 μl of icv LEM (not shown).

As shown in Fig. 2, a single injection of icv LEM (20 μl) produced a significant ($P < 0.001$) depression in plasma iron concentrations which was evident within 1 hr, reached an apparent nadir at 7 hr, and gradually started approaching, but did not achieve, control values by 24 hr. Plasma zinc concentrations also showed a significant ($P < 0.01$) decrease 3 hr postadministration icv with maximum depression occurring in 5 to 7 hr ($P < 0.001$). At the 24-hr period, plasma zinc was still significantly ($P < 0.01$) lower but had started to approach control

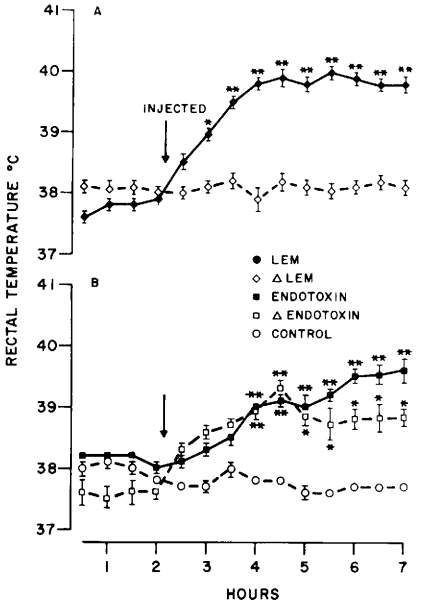

FIG. 1. Rectal temperatures induced by injecting 20 μl of LEM (◆, $n = 20$), ΔLEM (◇, $n = 10$) (panel A), endotoxin (■, 10 ng/20 μl, $n = 10$), Δendotoxin (□, 10 ng/20 μl, $n = 5$), or saline (○, $n = 5$) (panel B) icv into unanesthetized, unrestrained rats. Values are mean ± SE. *$P < 0.01$, **$P < 0.001$ vs ΔLEM or saline.

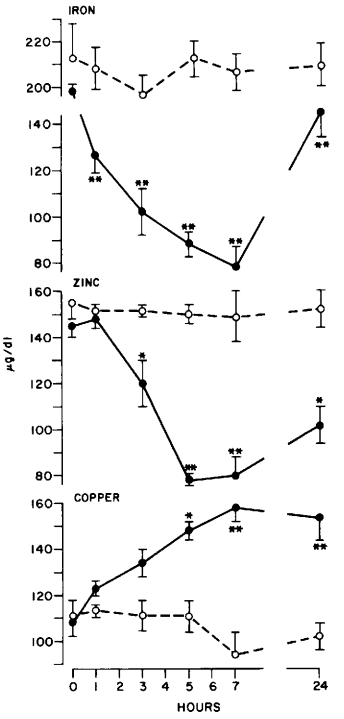

FIG. 2. Effect of icv LEM (20 μl) on plasma Fe, Zn, and Cu concentrations. Open circles represent control values of combined normal and ΔLEM rats. Closed circles represent LEM administration. Values are mean ± SE of five to eight rats. *$P < 0.01$, **$P < 0.01$ vs controls.

values (Fig. 2). Plasma copper concentrations were observed to be significantly elevated at the 5- ($P < 0.01$), 7- ($P < 0.001$), and 24-hr ($P < 0.001$) periods (Fig. 2). Heated LEM and saline given icv did not modify plasma iron, zinc, or copper concentrations from those of normal rats. Temperatures were also obtained from these rats after the icv injection and the results are similar to those presented in Fig. 1.

In order to determine the time sequence of the plasma α_2-MFP response, rats were injected icv with 10, 20, or 50 μl of LEM. All animals were bled from the orbital venous plexus at 0-, 5-, 24-, and 48-hr intervals after the injection; the plasma was assayed by radial immunodiffusion. The results are presented in Table I. Five hours after the injection of LEM, no significant change was seen. LEM in a icv dose of 10 μl failed to significantly elevate α_2-MFP at the 24- and 48-hr periods. Significant increases in plasma α_2-MFP were observed at icv doses of 20 and 50 μl at the 24- and 48-hr periods. Repeated orbital bleeding may have been responsible for the slight increase in α_2-MFP seen in animals given saline or heated LEM icv.

Two weeks after the initial icv administration of LEM, a 20-μl dose of saline was injected and the temperature was monitored for 8 hr to determine if hyperthermia occurred. Four days after the saline injection, each rat was injected sc with 1 μCi (0.1–0.2 μmole)/100 g body weight of the ^{14}C-nonmetabolizable amino acid 24 hr prior to use (1300 hr). At 0800 hr on the next day, rats were injected icv with 20 μl of LEM, heated LEM, or saline. At various time intervals (1, 3, 5, and 7 hr) after the icv injections, a 1-g sample of liver was removed from four individual rats at each time period for determination of hepatic concentration of [^{14}C]AIB. As shown in Fig. 3, LEM significantly increased the hepatic concentrations of [^{14}C]AIB. The increase was evident within 1 hr and remained elevated by the 7-hr period when compared to heated LEM values. Intraperitoneal injections of an equivalent volume of LEM (10, 20, and 50 μl) had no effect on the measured parameters (not shown).

Discussion. In the present study LEM (10–50 μl) was found to cause hyperthermia when injected into the lateral cerebral ventricle of the rat; these doses were inactive when administered ip or iv. In contrast, Kampschmidt and Upchurch observed rabbit LEM to produce hypothermia in rats following an ip injection. The fact that heat treatment abolished the hyperthermia produced by LEM but not that of endotoxin is good evidence that the LEM preparations were not contaminated by endotoxin (4). Additional evidence that endotoxin was not present in the LEM preparation was that LEM produced a more rapid onset of hyperthermia than did endotoxin.

Alterations in serum zinc, iron, and copper concentrations after ip injection of 1.0 ml of LEM have been well documented. This study demonstrates for the first time that icv injections of LEM cause a decrease in serum zinc and iron and increases in cop-

TABLE I. EFFECTS OF VARIOUS DOSES (10–50 μl) OF ICV LEM ON PLASMA α_2-MFP CONCENTRATIONS.[a]

		Hours			
μl	Treatment	0	5	24	48
10	Saline	0	0	3.0 ± 1.0	4.8 ± 0.5
	LEM	0.2 ± 0.1	0.4 ± 0.3	4.0 ± 2.0	6.0 ± 4.0
	ΔLEM	0.9 ± 0.4	8.0 ± 8.0	5.0 ± 3.0	4.0 ± 2.0
20	Saline	0	0	4.0 ± 4.0	8.0 ± 7.0
	LEM	0.4 ± 0.4	0.6 ± 0.5	24.2 ± 4.0**	24.2 ± 3.0*
	ΔLEM	3.3 ± 2.0	2.5 ± 2.0	6.6 ± 2.0	8.0 ± 8.0
50	Saline	0	0	2.0 ± 1.0	3.5 ± 3.0
	LEM	0.1 ± 0.1	7.0 ± 5.0	34.8 ± 10.0*	33.4 ± 12.0
	ΔLEM	0.9 ± 0.6	0.1 ± 0.02	6.1 ± 2.0	4.4 ± 1.0

[a] Values are mean ± SE of four to five rats in units per milliliter.
* $P < 0.05$ vs heated LEM; ** $P < 0.01$ vs heated LEM.

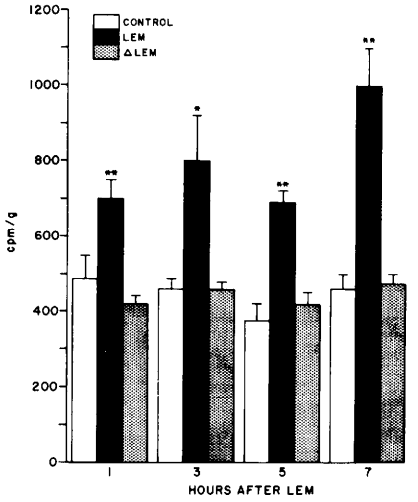

FIG. 3. Effects of icv LEM on hepatic concentration of [^{14}C]AIB. Rats were injected icv with either 20 μl of LEM, ΔLEM, or saline. Values are mean ± SE of four rats. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs controls.

per concentrations. Kampschmidt *et al.* (6) also observed changes in serum iron after intracisternal injections of 10 μl of LEM. These data are additional evidence of central nervous system (CNS) involvement in the action of LEM. Systemic (ip) administration of LEM has been shown to elevate the concentration of acute-phase globulins and stimulate an increased rate of transport of nonmetabolizable amino acids from the plasma to the liver (4) in rats. The present study demonstrates that an icv injection of LEM also leads to a heretofore undescribed CNS role in the elevation of an acute-phase protein (α_2-MFP) concentration and a marked hepatic intracellular accumulation of a nonmetabolizable amino acid. Thus, LEM can act via a CNS mechanism in stimulating the hepatic transport of amino acids and the synthesis of plasma α_2-MFP. In contrast, *in vitro* data suggest that LEM may act directly on liver cells to stimulate increased transport of amino acids (5).

The current studies have demonstrated that LEM in doses which are inactive ip can mediate certain effects on or through the CNS, thereby supporting the hypothesis that LEM may have a primary site of action in the CNS.

Summary. LEM obtained from glycogen-induced peritoneal exudates in rabbits were injected into the lateral cerebral ventricle of rats to determine if LEM had a primary site of action in the central nervous system. LEM injected icv in the dose of 10, 20, or 50 μl was observed to produce a rapid hyperthermia. Injection of heated LEM failed to cause a hyperthermic response. Endotoxin and heated endotoxin (20 ng/20 μl/rat) administered icv each produced a delayed hyperthermia as compared to LEM. The various doses of LEM were also observed to significantly decrease plasma iron and zinc, increase plasma copper, increase the synthesis of plasma α_2 acute-phase protein (α_2-MFP), and cause a flux of a nonmetabolizable amino acid ([^{14}C]AIB) to the liver. These observations suggest that LEM in doses which are inactive systemically can mediate certain effects on or through the central nervous system following icv administration.

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