Mediator of Hepatic Amino Acid Flux in Infected Rats[1] (36094)

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Little is known about mechanisms that control various alterations in the metabolism of a host infected with a virulent microorganism. A generalized decrease in the serum concentration of free amino acids during acute infection has been shown, through the use of a nonmetabolizable amino acid analog, 1-amino-cyclopentane-1-carboxylic acid (Cycloleucine), to result from a flux of amino acids into the liver. However, the mechanism leading to the observed amino acid flux has yet to be identified (1). Infection-related depressions of serum amino acids are associated in their timing with a decrease in serum zinc values and a rise in body temperature (2, 3). These latter responses have each been ascribed to the appearance in serum of an endogenous mediating factor (2, 3). Such a zinc-lowering factor has been detected in laboratory animals soon after the initiation of a bacterial or viral infection, experimental endotoxemia, or the administration of double-stranded synthetic polynucleotides (4). The mediator of zinc depression is a heat labile (inactivated at 90° for 30 min) substance similar in its action to the so-called "endogenous pyrogen" which is released by leukocytes obtained from peritoneal exudates (4).

The objectives of the present investigation were to determine if endotoxin, as well as other inflammatory materials, would also stimulate a flux of amino acids into the liver and whether or not an endogenous mediating factor was released into the serum of infected animals. Leukocytic extracts obtained from leukocytes were also assayed for their ability to stimulate amino acid flux into the liver.

Materials and Methods. Animals and treatment. Fisher Dunning rats (Microbiological Associates, Walkersville, MD) (weighing 150–200 g) were housed in a room maintained at 25–26° and lighted from 0600 to 1800 hr. The rats were fed an agar gel diet (5) which contained 18% casein. For 1 week before beginning a study, all rats were trained to consume their food between 0800 and 1600 hr. One day before its use in a study, each rat was injected subcutaneously with 1 μCi/100 g of body weight of ^{14}C-1-amino-cyclopentane-1-carboxylic acid (05.75 mCi/mmole, New England Nuclear, Boston, MA). Twenty-four hours later at 0800 hr the inoculum to be studied was injected intraperitoneally (ip) into the rat. At various times after inoculation, rectal body temperatures were obtained; the rats were anesthetized with 2-bromo-2-chloro-1,1,1-trifluoroethane (halothane) and blood was collected from an axillary-fold pouch after severing the brachial artery. The rats were killed by cervical dislocation; and their livers were perfused *in situ*, via the aorta, with 0.9% NaCl until cleared of visible blood. The whole liver was removed, weighed, and homogenized in 2 vol of cold 0.9 %NaCl.

Experimental regimen. In the first study, groups of 24 rats were inoculated with either 1 ml of sterile normal saline; 3×10^7 *Diplococcus pneumoniae*, type I, strain A5, as described previously (1); 3×10^7 heat-killed *D. pneumoniae* (56° for 30 min); 100 μg of lipopolysaccharide from *Escherichia coli* 0127:B8 (Difco Laboratories); or 100 μg of crystalline homopolymeric duplex of polyinosinic and polycytidylic acids (In·Cn) or polyadenylic and polyuridylic acid (An·Un) (Miles Laboratories). Three, 6, and 9 hr post-inoculation, 8 rats from each group were

[1] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

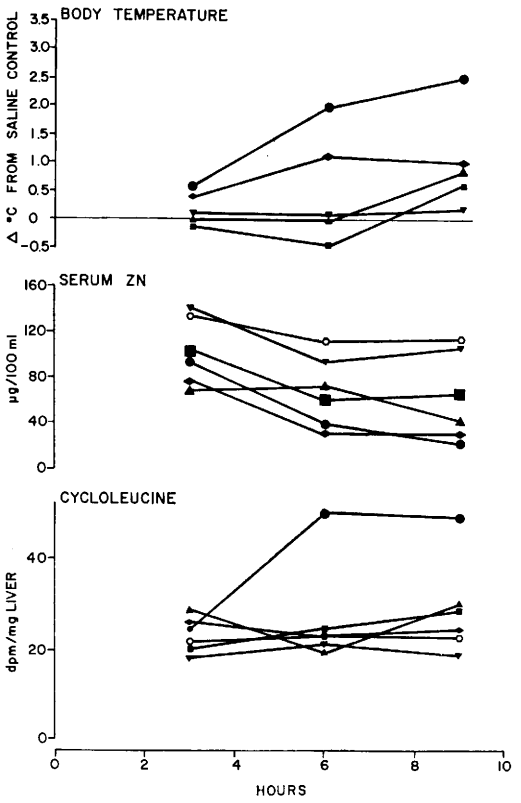

FIG. 1. Effects of live (●) and heat-killed (▲) *D. pneumoniae* (3×10^7); *E. coli* endotoxin (100 μg) (■); In·Cn (100 μg) (◆) An·Un (100 μg (▲); and 1 ml saline (○); on hepatic ^{14}C-cycloleucine content, serum zinc, and body temperature 3, 6, and 9 hr after an ip inoculation: Each point is the mean of 8 rats. The enlarged symbols indicate values that are significantly ($p<.01$) different from saline controls.

killed; and blood and liver samples were obtained.

In the second experiment, groups of 10 rats were inoculated ip with either 3×10^7 virulent *D. pneumoniae*, or 1 ml of sterile saline, and killed 2 hr later. The serum of each group was pooled, Millipore-filtered for sterility, and administered ip in 1.0-ml doses to 20 normal recipient rats. At 3 and 6 hr postinoculation, 10 recipient rats in each group were killed; and samples of blood and liver were obtained.

In the third series of studies, a crude preparation of endogenous mediator was obtained from rat leukocytes by a previously described procedure (4). Part of the endogenous mediator preparation was heat treated (30 min at 90°) and part was used without any modification. A "control" leukocytic preparation was obtained by subjecting peritoneal leukocytes to homogenization at 4° (6). Protein concentrations of the 3 preparations were determined by the method with varying doses of endogenous mediator, heat-treated endogenous mediator, control leukocytic homogenate, or 1.0 ml of saline. Eight rats per group were killed at 1, 2, 3, or 6 hr postinoculation; and samples of blood and liver were obtained.

Analytical procedures. Blood was centrifuged at 1500*g* for 15 min and serum was removed. Serum zinc concentrations were determined by atomic absorption spectrophotometry (2). A 1.0-ml sample of liver homogenate was added to 100 mg of sulfosalicylic acid, mixed and centrifuged at 1500*g* for 15 min. A 0.1-ml sample of filtrate was added to 11 ml of a scintillation cocktail containing 80 mg of butyl-PAB, 5 mg of PBBO, 10 ml of scintillation grade toluene, and 1 ml of general purpose solubilizer (Scintisol-GP, Isolab, Elkhart, IN). The sample was counted in a 3-channel Nuclear Chicago scintillation counter with external standardization. Counting efficiency for ^{14}C was 89%.

The data were analyzed by a computer for statistical significance. Group means were calculated and compared by Student's *t* test, and the difference between two means was considered significant at $p<.01$ under the null hypothesis.

Results. The effects of live and heat-killed *D. pneumoniae*, *E. coli* endotoxin, In·Cn, and An·Un on liver cycloleucine content, serum zinc, and body temperature are illustrated in Fig. 1. Only the live *D. pneumoniae* significantly increased the liver cycloleucine content above saline control values, with maximal effects at 6 and 9 hr postinoculation. At 3, 6, and 9 hr, all of the inocula, except heat-killed *D. pneumoniae*, significantly reduced serum zinc values below saline control concentrations with maximal effects between 6 and 9 hr. Body temperature was significantly elevated in rats injected with live *D. pneumoniae*, In·Cn, and An·Un, with maximal effect at the same time. *E. coli* endotox-

TABLE I. Effect of Serum from Infected and Control Rats on Cycloleucine Content of Liver and Serum Zinc.

Values shown are mean ± SEM.

Treatment of donor animals	No. of recipient animals	Hepatic cycloleucine (dpm/mg of liver) (hr): 3	6	Serum zinc (μg/100 ml) 3	6
Saline	10	29.3 ± 1.5	33.7 ± 0.8	120 ± 4	117 ± 4
Live *D. pneumoniae*	10	45.4 ± 2.9	39.1 ± 1.7	99 ± 4	80 ± 4

in produced a transient hypothermia at 6 hr and mild hyperthermia at 9 hr but the differences were not significant compared to values from saline controls.

Serum from rats inoculated with *D. pneumoniae* 2 hr before killing produced a significant increase in hepatic cycloleucine and marked depression in serum zinc concentrations of recipient animal compared to serum from rats inoculated with saline (Table I). Maximal effects on hepatic cycloleucine were noted in 3 hr after injection of serum from infected donors, while the greatest depressions of serum zinc were observed in 6 hr.

An injection of the crude endogenous mediator, which was released into saline by peritoneal leukocytes and contained 280 μg/ml of protein, stimulated a significant increase in hepatic cycloleucine content of recipient rats compared to saline-injected animals (Fig. 2). This increase was apparent 1 hr after the ip injection of the leukocytic mediator. For the next 2 hr, the cycloleucine content of the liver continued to accumulate at a logarithmic rate. Six hours after inoculation with the leukocytic mediator, the cycloleucine content of the liver was still significantly elevated above control values but was less than at 3 hr.

At 3 hr postinoculation the accumulation of hepatic cycloleucine was proportional to the logarithm of a dose of leukocytic mediator within the range of 30–100 μg of protein, (Fig. 3). Injection of 100–300 μg of mediator protein did not cause a further increase in cycloleucine content of liver.

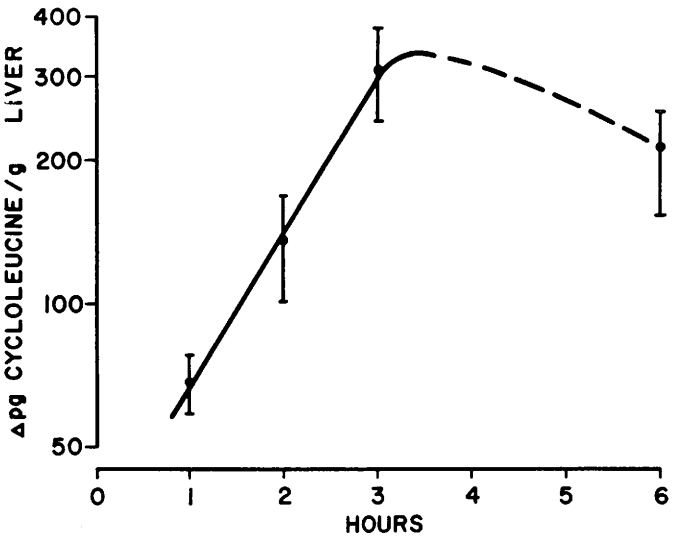

FIG. 2. The increase in hepatic cycloleucine content 1, 2, 3, and 6 hr after an ip inoculation of leukocytic mediator that contains 280 μg of protein: Each value is the mean × SEM of 8 rats and represents the difference between values found in saline controls and animals which received the leukocytic mediator.

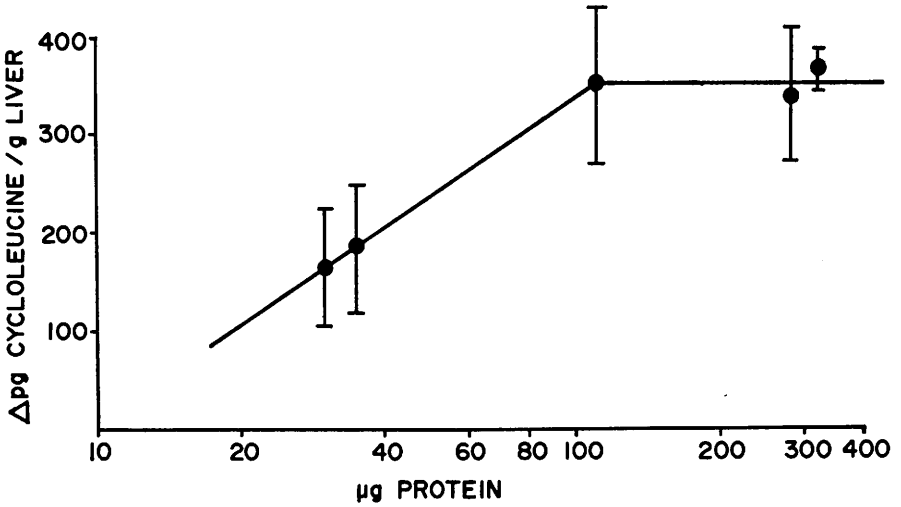

FIG. 3. Change in hepatic cycloleucine 3 hr after ip inoculation of various doses of leukocytic mediator: Each value is the mean ± SEM of 8 rats and represents the difference between values found in saline controls and animals which received the leukocytic mediator.

TABLE II. Cycloleucine Content of Liver 3 hr After Injection of Leukocytic Mediator, Heat-Treated Leukocytic Mediator, or Control Leukocytic Homogenate.
Values shown are mean ± SEM.

Treatment	No. of rats	Hepatic cycloleucine (dpm/mg of liver)
Saline	8	29.8 ± 1.4
Leukocytic mediator	8	61.6 ± 2.0
Heat-treated leukocytic mediator	8	33.6 ± 1.6
Control leukocytic homogenate	8	28.0 ± 1.8

Heat-treated leukocytic mediator and control leukocytic homogenate did not have any significant effect on hepatic cycloleucine compared to saline control values (Table II).

Discussion. As reported earlier, most of the infectious or inflammatory stimuli used herein will depress serum zinc and elevate body temperature (2, 4, 8, 9). Of this group, only the live *D. pneumoniae* significantly increased the cycloleucine content of liver. The movement of previously-equilibrated ^{14}C-cycloleucine into liver of infected animals is the result of a flux of amino acids from muscle to liver (1). Since cycloleucine is a nonmetabolizable amino acid analog which is slowly excreted by the rat (10), the increase in hepatic concentration of this amino acid is the result of stimulated rate of active transport into liver cells and across a concentration gradient.

Serum from rats injected with *D. pneumoniae* 2 hr before killing contained a mediator which stimulated the transport of cycloleucine into liver cells. A similar mediator of amino acid transport was released *in vitro* by peritoneal leukocytes. This mediator appeared to be a protein (or group of proteins) that were inactivated by heat. Such mediator preparations have exhibited pyrogenic activity (11, 12), as well as the ability to depress serum iron and zinc (4, 13, 14) and to increase the concentration of acute phase globulins in rat serum (6). A partially purified mediator extracted from material released into saline by peritoneal leukocytes has been shown to consist of at least six electrophoretically distinct proteins, only one of which provides the pyrogenic activity (15). Since only live *D. pneumoniae* significantly stimulated an increase in cycloleucine transport into liver and since there was a difference in the timing of maximal effects from the leukocytic mediator on amino acid flux and serum zinc depression, it may be hypothesized that a different protein intermediate is

responsible for mediation of each of the activities ascribed to the leukocytic preparation.

In noninfected and infected rats an increased flux of amino acids into liver is correlated with a stimulated rate of hepatic and serum protein synthesis (16–19). Thus, the leukocytic mediator, by stimulating a flux of amino acids into liver, may serve to provide necessary amino acid precursors and stimuli for the reported increased synthesis of serum a_1 and a_2 acute phase globulins (6).

Summary. Live *D. pneumoniae*, but not endotoxin or double-stranded polynucleotides, stimulated a flux of cycloleucine into liver. Sterile, 2-hr serum from *D. pneumoniae*-infected rats and secretions obtained from rat peritoneal leukocytes can also mediate a flux of cycloleucine into liver of normal recipient rats. The data were interpreted as evidence that one or more proteins synthesized and excreted by leukocytes can act as humoral intermediates in the stimulation of amino acid flux into liver tissue.

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