Vol. 88, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 14, 1979 Pages 327-334

COMPARISON OF ALBUMIN AND FIBRINOGEN BIOSYNTHESIS IN STIMULATED RATS AND CULTURED FETAL RAT HEPATOCYTES

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Received April 2,1979

Summary - Evidence is presented that cultured fetal rat hepatocytes, when incubated with a crude leucocytic extract derived from rabbits undergoing an inflammatory response, show a marked increase in fibrinogen production and a concomitant decrease in albumin production. Antibody binding to polyribosomes synthesizing fibrinogen and albumin shows the same inverse relationship in rats undergoing an artificially induced inflammatory response. These data demonstrate directly and unequivocally that during the acute phase (inflammatory) response, the biosynthesis of these two plasma proteins is inversely affected by a factor or factors acting directly on the liver.

The biosynthesis of hepatically derived plasma proteins has been studied in a number of systems (1-4). It is well documented that the plasma concentrations of fibrinogen, haptoglobin, C-reactive protein and other hepatically derived plasma proteins increase in response to physical trauma, infection or inflammation (for review see 5). On the other hand, plasma concentrations of albumin, transferrin and prealbumin have been reported to decrease during inflammation (6-7). These changes in protein levels together with the lowering of plasma iron and zinc levels (8-9) and an increased body temperature are referred to as the acute-phase response. Measurements of plasma and intracellular fibrinogen concentrations following the subdermal injection of turpentine to induce inflammation have shown that the changes in fibrinogen concentrations

This work has been supported in part by a J. W. McLaughlin Postdoctoral Fellowship to Randall G. Rupp and by grants from the National Institute of Health, HL16445 and a Research Career Development Award, HL00162 to Gerald M. Fuller.

0006-291X/79/090327-08$01.00/0

are due to changes in its biosynthesis (10). The decrease in albumin concentration during inflammation is therefore likely due to altered protein synthesis, however, definitive data have not heretofore been reported.

The precise nature of the molecular signals leading to the hepatic response during acute inflammation are not known. However, a crude leucocytic extract (CLE) from polymorphonuclear leucocytes of traumatized animals has been shown to mediate many of the events of the acute-phase response (11-12). The active factor(s) in the crude leucocytic extract has been called leucocytic endogenous mediator and/or leucocytic pyrogen (6,13). Recently, we have demonstrated that cultured primary fetal rat hepatocytes can be stimulated to increase fibrinogen production 2-3 fold over controls when crude leucocytic extract and cortisol are included in the incubation medium (14). It was therefore of interest to determine if the cultured hepatocytes responded to inflammatory factors by decreasing albumin synthesis.

By measuring the intracellular concentrations and by quantitating specific polyribosomes synthesizing fibrinogen and albumin during artificially induced inflammation, we have demonstrated a reciprocal relationship of these two proteins in vivo. In addition, we have shown a similar reciprocal relationship in the secretion of these two proteins by cultured hepatocytes incubated with crude leucocytic extract from traumatized animals.

METHODS AND MATERIALS

Antibodies to purified rat fibrinogen were produced in goats according to previously published procedures (15). Antibodies to rat plasma albumin were made in rabbits. Monospecific antibodies to each of these proteins were made by passing the sera from the immunized animal through an affinity column to which highly purified antigen had been covalently bound (16).

Iodination. Aliquots of monospecific antibodies (usually 3-5 mg/ml) were tagged with Na{^{125}I} using the methods described by Palacios (17). The tagged antibodies were made RNAse free by passing the iodinated protein through Sephadex G-100. The radiolabelled, RNAse free monospecific antibodies were concentrated and stored at -20°C.

Polysomal analysis: Polysomes were isolated from rat liver by centrifugation of the post mitochondrial supernatant through a discontinuous gradient as previously described (15). Purified polysomes were then incubated with

tagged antibody and analyzed using linear sucrose gradient procedures. Aliquots of the gradient were taken and the radioactivity of each tube determined using a Packard scintillation spectrometer.

Quantitative Immunological Assay. Immunochemical quantitation of fibrinogen and albumin in the cytosol and in culture medium utilized an enzyme-linked immunosorbent assay (18).

Cell Cultures. Hepatocytes taken from 17 day rat fetuses were cultured according to our previously described procedures (14).

Crude Leucocytic Extract. Polymorphonuclear leucocytes were obtained from peritoneal exudates of rabbits by the procedure of Pekarek et al. (19). Briefly, the leucocytes were collected by centrifugation, washed, suspended to 10^8 cells per ml of pyrogen-free physiological saline and incubated at 37°C for four hours. After the incubation, cells and debris were removed by centrifugation and the supernatant was termed crude leucocytic extract (CLE). Total crude leucocytic extract protein was determined by the method of Lowry et at. (20).

RESULTS

The hepatocellular concentrations of fibrinogen and albumin at various times subsequent to turpentine induced inflammation are shown in Figure 1. At 10 hours post injection, increased intracellular levels of fibrinogen were apparent. A three-fold increase occurred 24 hours post injection and then re-

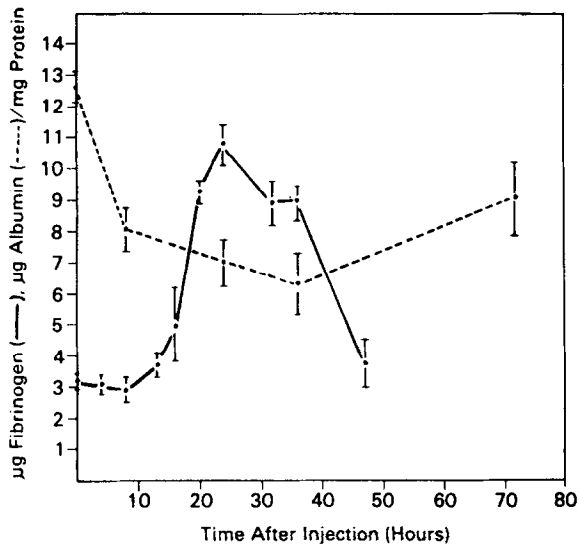

Figure 1. Intracellular levels of fibrinogen and albumin following turpentine-induced inflammation. The concentration of fibrinogen and albumin in the postmicrosomal supernatant (S_3) for each time point was determined by the enzyme-linked immunosorbent assay as previously described (18). Each point represents the average of triplicate analysis in 2-3 animals (± SEM).

turned to nearly normal by 48 hours. In contrast to the increase in intracellular fibrinogen, concentrations of albumin decreased to (ca. 40%) of normal levels within 8 hours following the injection. The maximum decrease of 50% occurred between 24-36 hours post injection. By 72 hours, albumin had returned to 73% of normal values.

In order to determine if the increase in fibrinogen and decrease in albumin resulted from changes in protein synthesis and not merely to release from sequestered storage sites, total liver polyribosomes were isolated and analyzed. Polyribosomes specifically involved with fibrinogen and albumin synthesis were identified using $\{^{125}I\}$ labelled anti-fibrinogen and $\{^{125}I\}$ anti-albumin, respectively. Figures 2A and 2B show the polyribosome profiles

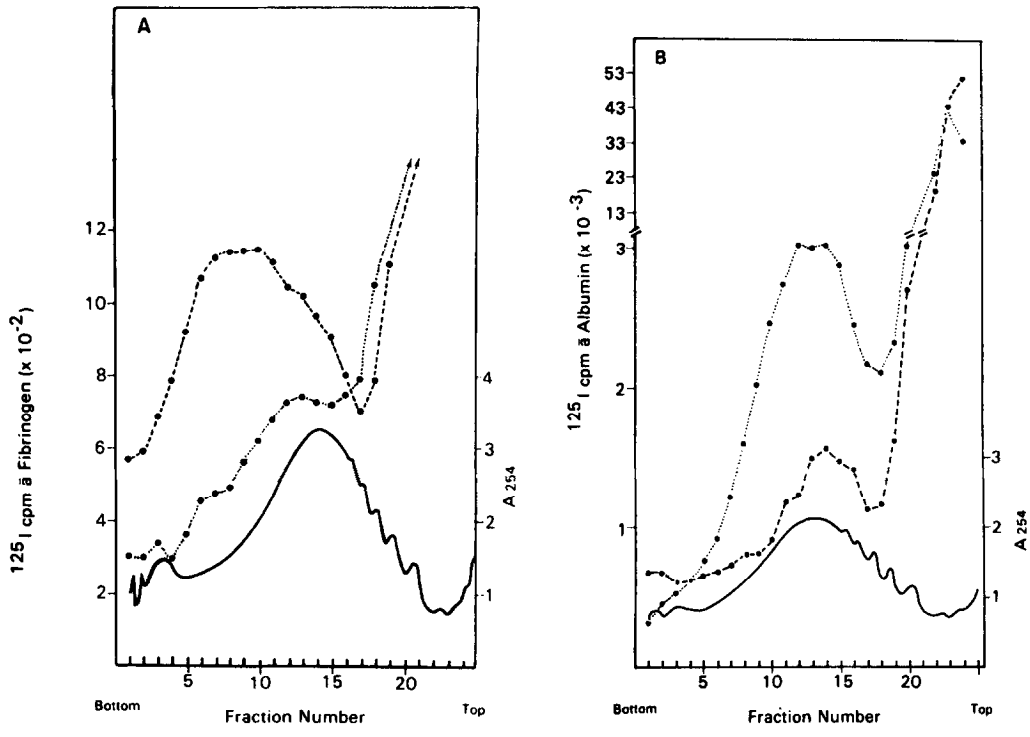

Figure 2. Identification of polyribosomes synthesizing fibrinogen and albumin during the acute inflammatory response. Rat liver polysomes were obtained 24 hours after a subdermal injection of turpentine (----) or saline (····). Five A_{254} units of polysomes in 0.3ml (_____) were incubated on ice for 30 minutes with (A) 15µg of $\{^{125}I\}$ anti-fibrinogen or (B) $\{^{125}I\}$ anti-albumin. They were then centrifuged on 17-52% linear sucrose gradients, the A_{254} recorded and the fractions collected and counted on a Packard gamma counter.

and specific radioactive labelling of those polyribosomes engaged in the synthesis of fibrinogen and albumin isolated from the livers of control and stimulated rats. An increase in the amount of radioactivity is taken to mean an increase in the number of available nascent chains and hence an increase in protein synthesis (10). After analyzing the polyribosome bound radioactivity from 4-5 rat preparations by the method of Kwan and Fuller (10) (data not included), it was determined that the number of polyribosomes specifically labelled with {^{125}I} anti-fibrinogen increased 200-300% following turpentine injection; whereas, those labelled with {^{125}I} anti-albumin, decreased approximately 40-50%.

The effect of three different crude leucocytic extract preparations on fibrinogen and albumin production in cultured fetal rat hepatocytes is shown in Table 1. There was a 69-95% increase in fibrinogen over control when the cells were incubated with 50µg/ml of crude leucocytic extract. We have previously shown that crude leucocytic extract in the absence of cortisol has no effect on fibrinogen synthesis in cultured primary fetal hepatocytes (14). The effect of crude leucocytic extract on albumin production by cultured fetal hepatocytes is also shown in Table 1. There is consistently a decrease in the albumin concentration of the medium after incubation of the cells with crude leucocytic extract and cortisol. Fifty micrograms per ml of crude leucocytic extract routinely caused a 30-50% reduction in albumin secretion. However, as can be seen from the table, albumin was occasionally decreased as little as 6% even though fibrinogen was increased 95%.

DISCUSSION

We have previously demonstrated that a crude leucocytic extract from traumatized animals can act directly on cultured, primary fetal rat hepatocytes to increase fibrinogen biosynthesis--one event of the acute-phase response. It was of interest to conclusively show that the reported *in vivo* reduction in plasma albumin concentrations during inflammation were due, at least in part, to a decrease in albumin biosynthesis. In addition we felt it important

Table 1

Effect of Crude Leucocytic Extract on Fibrinogen and Albumin Synthesis in Cultured Fetal Rat Hepatocytes

Experiment	μg Fibrinogen / 100μg cell protein	μg Albumin / 100μg cell protein	% Increase In Fibrinogen Over Control	% Decrease In Albumin Over Control
No. 1				
Control	1.83 ± .17	10.13 ± .65	-	-
CLE_1	3.56 ± .09	9.55 ± .94	95%	6%
No. 2				
Control	1.61 ± .16	11.79 ± 1.31	-	-
CLE_2	3.03 ± .26	6.35 ± .56	88%	46%
CLE_3	2.72 ± .12	7.28 ± .62	69%	38%

Hepatocytes were obtained as described (14) and plated at 2 x 10^6 cells per well in Costar 24 well tissue culture plates. Twenty-four hours later, the cells were washed with Williams essential medium supplemented with 10% fetal bovine serum after which 1 ml of medium containing 10^{-6}M cortisol and 50μg of crude leucocytic extract (CLE) was added to each well and the incubation continued for twenty-four hours at 37°C. At the end of the twenty-four hours the medium was collected and assayed for fibrinogen and albumin using the enzyme-linked immunosorbent assay. Each well of cells was rinsed, solubilized with 2N NaOH and total cellular protein determined by the procedure of Lowry et al. All the data are averages of four individual treatments ± SEM. The subscripts indicate three different CLE preparations.

to demonstrate that the crude leucocytic extract can act on cultured fetal hepatocytes to mimic the in vivo response by decreasing albumin synthesis.

As was shown in the results, there is a reciprocal relationship between fibrinogen and albumin production in vivo during inflammation as well as in cultured fetal hepatocytes in response to leucocytic factors from stimulated rabbits. Previous studies from our laboratory have shown that sera from surgically traumatized or turpentine stimulated rats contain non-dialyzable factors which can stimulate fibrinogen production in cultured fetal hepatocytes (14). It seems likely that the response of the cultured hepatocytes to crude leucocytic extract and the response of the intact liver (in vivo experiments) during inflammation are similarly mediated.

While the data in this report clearly demonstrate the reciprocal biosynthetic events between fibrinogen and albumin, it in no way suggests a molecular mechanism. The elucidation of the precise involvement of the crude leucocytic extract in the synthesis of these important hepatic proteins awaits further study. Since cultured fetal hepatocytes mimic the in vivo response to inflammation (at least in regard to fibrinogen and albumin production), they will be used to assay for the presence of inflammatory factors and to investigate their molecular mechanism of action.

ACKNOWLEDGMENT

We wish to thank Robin Miller for excellent technical assistance.

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