Synthesis of Fibronectin by the Isolated Perfused Rat Liver

By Michael R. Owens and Catherine D. Cimino

The major site(s) of synthesis of plasma fibronectin is unknown. Using the isolated perfused rat liver and anti-rat fibronectin antiserum, we have measured net hepatic synthesis of fibronectin during 10-hr perfusion periods. We calculated the circulating plasma fibronectin pool of a 195-g rat with body surface area of 300 sq cm to be 3.3 mg; net hepatic synthesis of 3.76 ± 1.0 mg/300 sq cm body surface area of the rat liver donor was seen in liver perfusions of 10 hr. When the hormones cortisol and insulin were added to the liver perfusate, net synthesis of 5.05 ± 0.82 mg/300 sq cm was seen. Synthesis of two known acute phase reactant proteins, fibrinogen and alpha-2 acute phase globulin, was also substantially greater in the presence of cortisol and insulin. Plasma fibronectin levels measured in intact rats that had abscess produced by subcutaneous turpentine injection were 0.57 ± 0.05 mg/ml compared to 0.38 ± 0.02 mg/ml in normal control animals. Our studies indicate that hepatic synthesis contributes substantially to the plasma fibronectin pool and show that such synthesis is enhanced by the hormones cortisol and insulin. In the intact rat, inflammation was associated with elevated levels of plasma fibronectin.

FIBRONECTIN, also known as cold-insoluble globulin, is an alpha-2 globulin that is found in association with cell surfaces and also as a circulating plasma protein. There are physical and perhaps functional differences between the two types of fibronectin, but the two forms are immunologically very similar. Fibronectin is present in many body tissues, but the major site(s) of synthesis of plasma fibronectin remains unknown. Production of cell surface fibronectin by fibroblasts and endothelial cells in culture has been reported, and it has been suggested that this cell surface form is the precursor of plasma fibronectin. Fibronectin has also been detected by immunofluorescent techniques in fetal rat hepatocytes, but it is not known whether the factors contribute significantly to plasma levels of fibronectin. Factors regulating plasma fibronectin synthesis have not yet been defined; in particular, the hormones cortisol and insulin, known to regulate synthesis of the acute phase proteins, have not been studied.

The system of perfusion of the isolated rat liver has proven to be an excellent tool for studies of protein synthesis. The hormones cortisol and insulin, when added to the liver perfusion system, lead to an increase in synthesis of certain liver secretory proteins known as "acute phase reactant proteins," while in the intact animal, inflammation enhances synthesis of these same proteins.

In the study described here, the isolated perfused rat liver was utilized to investigate the role of the liver in plasma fibronectin synthesis as well as to study effects of insulin and cortisol on synthesis. The effect of inflammation on plasma fibronectin levels was also studied in intact rats with a sterile abscess secondary to subcutaneous turpentine injection.

MATERIALS AND METHODS

Liver donors were normal, fed, Holtzman rats weighing 350–400 g. Hepatectomy was performed while the animal was under diethyl-ether anesthesia.

Perfusion of the isolated rat liver was carried out as previously described. The liver perfusate consisted of 38 ml washed bovine red cells (GIBCO, Grand Island, N.Y.) suspended in 50 ml Krebs-Ringer bicarbonate buffer containing bovine serum albumin 3 g/dl, glucose 100 mg, penicillin 3000 U, streptomycin-HCl 3 mg, and heparin 5000 U. Sufficient Ringer solution was added to bring the perfusate volume to 100 ml. In control perfusions, a constant infusion consisting of 18 ml Ringer solution containing glucose 500 mg, amino acids 320 mg, penicillin 3000 U and streptomycin-HCl 3 mg was added to the perfusate at a rate of 1.5 ml/hr. In “full supplementation” experiments, to stimulate synthesis of the acute phase reactant proteins, the constant infusion included cortisol 5 mg and insulin 6.8 U; the hormones insulin (5.1 U) and cortisol (5 mg) were also added directly to the perfusate at the outset. In all experiments, the pH of the perfusate was maintained constant at 7.40 by an infusion of 1.0 M NaHCO3 from a Radiometer (The London Co., Cleveland, Ohio) titrator and autoburette. At the start of each perfusion, the first 10 ml of perfusate to pass through the liver was collected and discarded to eliminate washout of preformed rat plasma proteins. In selected experiments to block protein synthesis, puromycin (15 mg) was added to the perfusate at the outset and an additional 7.5 mg was added by constant infusion. All perfusion experiments were run for a period of 10 hr, and samples of the liver perfusate were withdrawn for assay at 2 hr intervals.

To elicit the “acute phase response” in vivo, 5 rats were injected with turpentine (1 ml) subcutaneously in the flank. Forty-eight hours later, these rats were bled by aortic puncture into syringes containing Na citrate, 0.17M (whole blood:anticoagulant, 9:1 v/v), and fibronectin concentrations in the individual plasmas were compared to those from 5 normal rats bled the same way.

Specific antiser to rat fibrinogen and alpha-2 acute phase globulin were prepared as described previously and used for measurement of these proteins in perfusate samples by the single radial immunodiffusion assay of Mancini et al., as modified by Fahey and McKelvey. Antiserum to rat fibronectin, as well as the purified protein used as standard, was purchased from Calbiochem (La Jolla, Calif.).

From the Department of Medicine, University of Rochester, School of Medicine and Dentistry, Rochester, N.Y.

 Submitted April 13, 1981; accepted February 8, 1982.

Address reprint requests to Michael R. Owens, M.D., Department of Medicine, St. Mary’s Hospital, 89 Genesee Street, Rochester, N.Y. 14611.

© 1982 by Grune & Stratton, Inc.

Blood, Vol. 59, No. 6 (June), 1982

1305
The mean cumulative synthesis of fibronectin in control and full supplementation liver perfusions of 10-hr duration is shown in Fig. 1. Under full supplementation conditions, greater synthesis of fibronectin was seen, 5.05 ± 0.82 mg/300 sq cm body surface area of the rat liver donor compared to 3.75 ± 1.00 mg/300 cm² in control perfusions. This increase in net synthesis was apparent by 6 hr of perfusion. When puromycin was added to 4 perfusions to block protein synthesis, production of fibronectin was markedly decreased compared to control experiments, but slight increases were observed over the final 6 hr of perfusion. This increase in fibronectin, in the presence of sufficient puromycin to effectively inhibit protein synthesis by the perfused rat liver, is in contrast to the effects on fibrinogen synthesis seen in Fig. 2. Synthesis of fibrinogen was effectively blocked in those perfusions containing puromycin, and the effect of hormones on synthesis of this known acute phase reactant protein are apparent in the figure. Cumulative synthesis of alpha-2 acute phase globulin in control and full supplementation perfusions is shown in Fig. 3. This protein was not detectable in control perfusions, but a mean of 1878 ± 297 U was synthesized under full supplementation conditions.

Plasma from rats that had sterile abscess produced by turpentine injection had significant elevations of fibronectin, 0.57 ± 0.05 mg/ml compared to 0.38 ± 0.02 mg/ml for control animals (Fig. 4). Alpha-2 acute phase globulin levels were also markedly elevated in
Plasma fibronectin levels vary considerably in pathologic states commonly associated with elevations of acute phase plasma proteins, and it is apparent that while some chronic disorders may be associated with increased plasma fibronectin, acute trauma may result in rapid depletion of plasma fibronectin. Increased fibronectin levels have been found in patients with chronic inflammatory disorders such as rheumatoid arthritis, but no significant changes were observed in a group of myocardial infarction patients. Increased as well as decreased plasma fibronectin levels have been observed in sera from patients with carcinoma. In states of acute trauma, rapid depletion of plasma fibronectin may be seen, such decreases have been observed following burn injury and in disseminated intravascular coagulation. Our observations utilizing the isolated perfused rat liver have been confined to the effects of certain hormones—cortisol and insulin—on synthesis of fibronectin. The data are not affected significantly by distribution or consumption of the protein, whereas levels in vivo reflect all of these factors.

Our studies also provide quantitative information on the possible role of hepatic synthesis in plasma levels of fibronectin. The rat liver donor body surface of 300 sq
cm corresponds to a body weight of 195 g and a plasma volume of 8.8 ml. The plasma fibronectin level in pooled rat plasma was found to be 0.38 mg/ml, which is slightly lower than previously reported values. At this level of 0.38 mg/ml, a 195-g rat would have a circulating plasma pool of 3.3 mg fibronectin. Cumulative synthesis in 10 hr of 3.75 mg in control perfusions and 5.05 mg in full supplementation perfusions indicates that the liver is capable of contributing substantially to the plasma fibronectin pool.

The increasing levels of fibronectin observed in perfusions containing puromycin may indicate some continued release or secretion of this protein by the perfused rat liver. The quantity of puromycin added has been shown to effectively inhibit protein synthesis by the perfused rat liver, and the effects we observed on fibrinogen synthesis confirm this.

Our studies do not define the specific cell type(s) involved in fibronectin synthesis, since the liver contains endothelial cells known to synthesize fibronectin, in addition to hepatocytes; however, hepatocytes comprise approximately 78% of liver volume in the adult rat and nonhepatocytes approximately 6%. The ability of fetal hepatocytes to synthesize fibronectin has been previously documented by immunofluorescent studies, and there is evidence suggesting that dexamethasone greatly enhances such synthesis.

ACKNOWLEDGMENT
We thank Bonnie DeConinck for her assistance in preparation for this manuscript.

REFERENCES
Synthesis of fibronectin by the isolated perfused rat liver

MR Owens and CD Cimino