Calmodulin may decrease cell surface sialic acid and be involved in the expression of fibronectin during liver regeneration

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The decrease of sialic acid in plasma membrane glycoproteins and the expression of cell surface fibronectin were studied during the pre-replicative phase of liver regeneration. The aim of this study was to correlate these cell-surface events to the intracellular surge of calmodulin observed a few hours after partial hepatectomy. The fact that calmodulin decreased the specific activity of UDP-*N*-acetyl-D-glucosamine 2′-epimerase, a key regulatory enzyme in the biosynthesis of glycoprotein sialic acids, and that trifluoperazine prevented the desialylation indicates that the membrane desialylation is a calmodulin-dependent process. On the other hand, Western blotting using anti-rat fibronectin antibody in trifluoperazine-treated animals suggests that calmodulin may also be involved in the surface expression of fibronectin in regenerating hepatocytes.

Liver regeneration *Fibronectin* *Sialic acid* *Calmodulin* *Cell surface*

1. INTRODUCTION

The transition of quiescent hepatocytes into the cell cycle is achieved by a series of events that deliver the cell to the S phase and towards subsequent cell division. The transitional phase is accompanied by activating the transcription of cell cycle genes, increasing amino acid uptake, reducing protein degradation, activating ornithine decarboxylase, and laying down additional basal lamina and others [1]. These changes are followed by a wave of cAMP accumulation and an intracellular surge of calmodulin [2]. Some early intracellular changes will have effects at the level of the plasma membrane acting on enzyme activities, modulating the affinity of the receptors or affecting the shape and/or adhesion of the hepatocytes. Therefore, it is apparent that the cell surface is intimately involved as a receptor structure for growth factors and hormones, as well as a transducing organelle for the extracellular signals.

We have previously described that during the pre-replicative phase of liver regeneration there was a decrease in sialic acid [3] and an increased amount of fibronectin at the sinusoidal plasma membrane of regenerating hepatocytes [4]. Our goal is to relate these two events to the calmodulin surge using the specific calmodulin blocker trifluoperazine. Preliminary work indicates that this agent induces delayed DNA synthesis [5], and may affect membrane protein desialylation when injected 4 h after partial hepatectomy [6].

We have now studied the decrease in sialic acid in more detail and report that calmodulin evidently is involved in the regulation of desialylation of sinusoidal plasma membrane glycoproteins. It may also be involved in fibronectin expression/shedding on this aspect of the hepatic cell.

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2. EXPERIMENTAL

Male Sprague-Dawley rats weighing 250–300 g, maintained on a standard rat diet, were used for all experiments. Partial hepatectomies were performed between 8 and 10 a.m. Surgery was carried out using the procedure of Higgins and Anderson [7]. Ligation and excision of the median and left lateral lobes of the liver constituted partial hepatectomy (70%).

Sinusoidal plasma membranes were isolated [8], proteins separated on 8% polyacrylamide gels by electrophoresis in the presence of SDS and 2-mercaptoethanol [9], and blotted onto nitrocellulose filters [10]. A rabbit anti-rat fibronectin antiserum prepared by immunization with purified rat plasma fibronectin [11] was used followed by incubation with ^{125}I-protein A.

Sialic acid was measured by the method of Aminoff [12] and the specific activity of UDP-*N*-acetyl-D-glucosamine 2′-epimerase was determined from the cytosol [13] by the procedure of Spivak and Roseman [14] and that of Reissig et al. [15] for mannosamine detection.

For immunofluorescence, livers were fixed by portal perfusion with 3% paraformaldehyde in phosphate-buffered saline [16] and after fixation pieces of tissue were dehydrated, embedded in paraffin and sectioned [17]. We have used the indirect immunofluorescence method according to Sternberger [18]. Anti-fibronectin antiserum was incubated overnight at 4°C, and bound antibody was detected by incubation with swine anti-rabbit IgG conjugated to fluorescein isothiocyanate (DAKO).

Calmodulin was isolated from bovine brain by the method of Guerini et al. [19].

Protein was determined by the method of Lowry et al. [20].

3. RESULTS

Fig.1 shows the changes in the sialic acid bound to the sinusoidal plasma membrane at 6 and 12 h after partial hepatectomy. To examine if the decrease in plasma membrane sialic acid was due to a lower biosynthetic activity we measured UDP-*N*-acetyl-D-glucosamine 2′-epimerase, a key regulatory enzyme involved in the conversion of *N*-acetylglucosamine to *N*-acetylmannosamine, a sialic acid precursor [21].

Table 1 shows that UDP-*N*-acetyl-D-glucosamine 2′-epimerase activity was significantly decreased during the first hours of the regenerating process. To relate the decrease of sialic acid and the decreased 2′-epimerase activity to the intracellular surge of calmodulin, we used

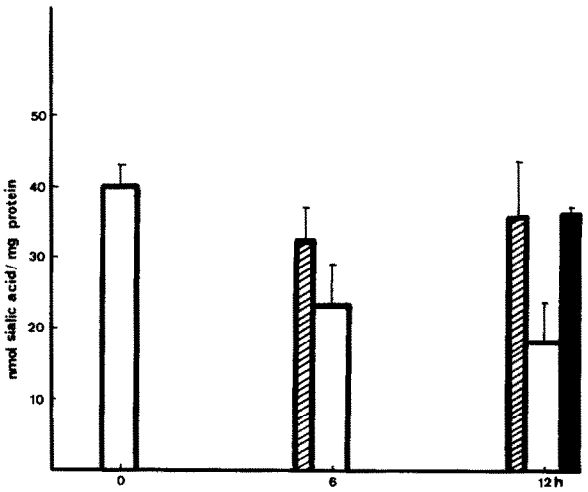

Fig.1. Content of sialic acid bound to the sinusoidal plasma membrane at (0) control, 6 h and 12 h after partial hepatectomy (open bars). Hatched bars represent the content of sialic acid of sham-operated animals and the closed bar denotes the amount seen after treatment with trifluoperazine (60 mg/kg) injected 4 h after the operation, and studied at 12 h of the regenerative process.

Table 1

Specific activity of UDP-*N*-acetyl-D-glucosamine 2′-epimerase from control and partially hepatectomized animals

	Spec. act. (nmol product/ 30 min per mg protein)	(*n*)	% decrease
Control	45.12 ± 3.29	(7)	–
6 h	30.33 ± 0.88	(4)	33.2
12 h	33.32 ± 2.29	(4)	26.1

No significant changes in the specific activity were found in the sham-operated animals compared to non-operated control animals at 6 and 12 h

Table 2

Effect of Ca^{2+}-calmodulin on UDP-*N*-acetyl-D-glucosamine 2′-epimerase activity

	Enzyme activity (nmol product/30 min per mg protein)		
	0.5 μM CaM	1 μM CaM	5 μM CaM
Control 45.12 ± 3.29 (5)	32.55 ± 4.82 (6)	32.7 ± 4.13 (5)	32.80 ± 2.8 (6)
Per cent decreased activity	27	27	27

CaM, calmodulin. In all assays 0.03 mM Ca^{2+} was added together with CaM

trifluoperazine, a calmodulin blocker. Fig.1 shows that when this agent was injected at 4 h after operation it prevented the decrease in sialic acid seen at 12 h. Further evidence for the involvement of calmodulin was obtained when it was included in the in vitro enzymatic assay. Table 2 shows that over the range 0.5–5 μM calmodulin a 27% decrease in activity occurred.

Using quantitative Western-blot analysis (fig.2) we studied the expression of fibronectin at the sinusoidal plasma membrane. At 12 h after partial hepatectomy a significant increase of fibronectin was observed, but when trifluoperazine-treated animals were examined for fibronectin expression at this plasma membrane region no changes were detected.

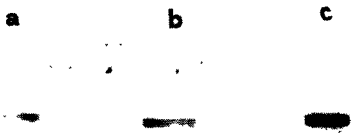

Fig.2. Visualization by Western blotting of fibronectin from rat liver plasma membrane. 40 μg of the sinusoidal plasma membrane isolated as described in [8] was used for immunoblotting. (a) Control (unoperated) rats, (b) 12 h hepatectomized rats and treated with trifluoperazine, (c) 12 h hepatectomized rats.

4. DISCUSSION

At cell division major cellular structures are reorganized. Microtubules and actin filaments are directly engaged in the mitotic process and outside the cell the extracellular matrix plays a major part in growth and development of the regenerating tissue. If cell adhesion is understood as a transmembrane process then one can expect that changes in the adhesion might be controlled by intracellular signals.

The Ca^{2+}-calmodulin complex is involved in several cellular processes such as the polymerization of microtubules and microfilaments and in desmosome organization, i.e. it is involved in the arrangement of the cytoskeleton [22].

On the other hand, Dennis et al. [23] suggest that an increase in sialylation of metastatic tumor cell surfaces can result in decreased attachment to basement membrane proteins collagen type IV and fibronectin, predisposing the tumour cells to increased mobility and decreased growth control by substratum contact.

Regenerating hepatocytes exhibit less sialic acid at the cell surface, and we have studied whether this change and increase in fibronectin could be related to the intracellular surge of calmodulin at the end of the pre-replicative phase of liver regeneration.

From the data obtained it is clear that

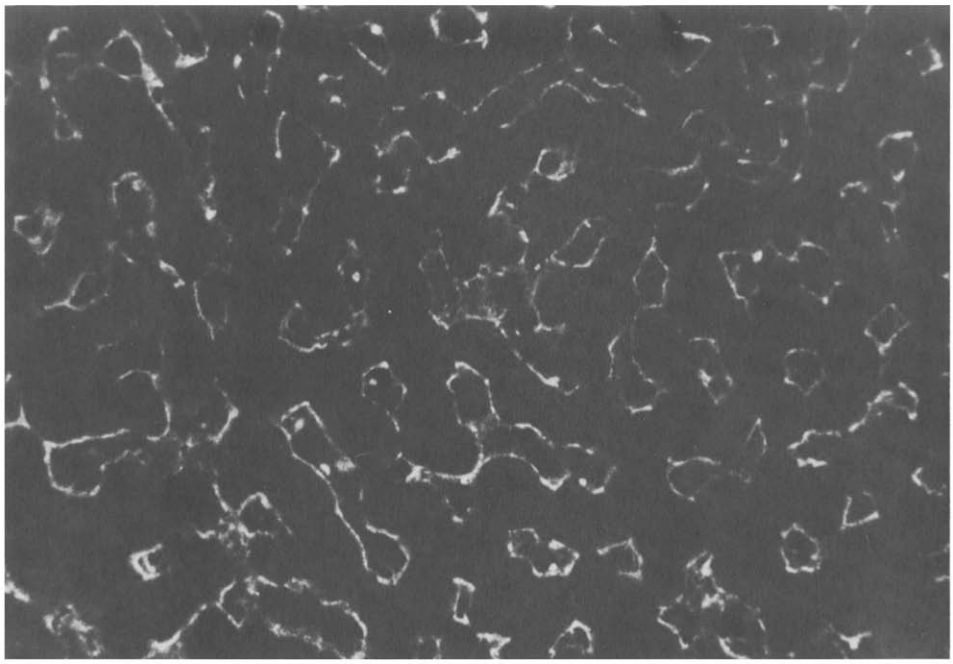

Fig.3. Location of fibronectin in the hepatic tissue by indirect immunofluorescence microscopy with a polyclonal anti-fibronectin antibody. The micrograph (× 400) shows that the fluorescence was mainly concentrated at sinusoids.

trifluoperazine, injected 4 h after the partial hepatectomy, prevented the decrease in sialic acid of the plasma membrane glycoproteins, and that this phenomenon can at least partially be explained by the inhibition of UDP-*N*-acetyl-D-glucosamine 2′-epimerase due to the intracellular surge of calmodulin.

Furthermore, the Western-blot analysis shows that the trifluoperazine injected 4 h after partial hepatectomy prevented the increase of fibronectin normally occurring at 12 h of regeneration.

Since most of the cell-surface hepatic fibronectin is located at the sinusoids (fig.3) it appears that the deposition of fibronectin at the sinusoidal face of the hepatocyte could have some implication for subsequent cell division, such as providing attachment for proper tissue organization during liver regeneration. This interaction could also provide a stimulus for proliferation of liver cells.

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