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Reduced levels of sialic acid in the plasma membrane during hepatocellular proliferation

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When rats were infused with a solution containing triiodothyronine, amino acids, glucagon and heparin (solution A) the hepatocytes increased DNA synthesis and decreased plasma membrane sialic acid. In order to study whether the reduced levels of sialic acid in the plasma membrane were associated with hepatocyte proliferation, different mixtures of three components of solution A were infused into rats and the DNA synthetic activity as well as the sialic acid content measured. Results reported here show a correlation between DNA synthetic activity and sialic acid reduction suggesting that the decrease in the plasma membrane sialic acid can be a pre-replicative step associated to cell proliferation.

Partial hepatectomy induces liver cells to proliferate. In the remaining liver tissue, proliferative activation triggers a wave in DNA synthesis starting at 16 h and peaking at 24 h after surgery [1,2]. Many cellular changes occur during the pre-replicative phase of liver regeneration after a partial hepatectomy [3–5]. Among them, we have previously reported a decrease in sialic acid bound to the hepatocyte plasma membrane starting at 6 h and presenting minimum values at 12 h post-hepatectomy [6]. This decrease in the plasma membrane sialic acid can be partially explained by a diminished activity of the UDP-*N*-acetyl-D-glucosamine 2′-epimerase, a key enzyme of the biosynthetic pathway of sialic acid [7,8]. Moreover, the decreased activity of this enzyme seems to be produced by the surge of cytosolic calmodulin observed between 6 and 12 h after partial hepatectomy [9,10] since it was shown that calmodulin inhibited the specific activity of this enzyme [7]. In order to establish if the reduced levels of sialic acid in the plasma membrane were a pre-replicative step associated to hepatocyte proliferation it seems interesting to know whether a decrease in sialic acid at the plasma membrane also occurs in the hepatocytes proliferatively activated by other stimuli different to partial hepatectomy. It is also important to correlate DNA synthesis with the reduction of sialic acid in the plasma membrane. In this paper we have measured the content of sialic acid in the hepatocyte plasma membrane from rats infused with a solution containing triiodothyronine, amino acids, glucagon and heparin (solution A) that produces a proliferative activation similar to that produced by a partial hepatec-

Abbreviations: T, triiodothyronine (T_3); A, amino acids; G, glucagon; H, heparin; TAGH, solution A.

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tomy [11]. Furthermore, the relationship between sialic acid content and DNA synthetic activity after the infusion of several different mixtures of three components of solution A have been studied.

Rat liver plasma membranes derived from the sinusoidal domain were prepared essentially by the method of Wisher and Evans [15] and described in detail in [16]. The membranes were carefully washed in 10 mM Tris-HCl (pH 7.5) to remove completely the sucrose, and the amount of sialic acid was quantified using the method of Aminoff [12] with the correction factor introduced by Warren [17]. However, since it has been shown that lipid adducts generated by the mild acid hydrolysis can interfere with the sialic acid determination [19,20] and considering that an important increase in the lipid content occur in this early stages of liver regeneration [21], a lipid extraction of the isolated plasma membrane fractions was carried out [22] to assess the reliability of sialic acid measurements. No significant differences were found compared with the unextracted membranes neither in control or in the membranes of hepatectomized animals.

The DNA synthetic activity was assayed measuring the radioactivity incorporated into DNA after the injection of [^{3}H]thymidine (0.5 μCi/g body weight) 1 h before the animals were killed [13]. Protein was determined by the method of Lowry et al. [14].

The intravenous infusion of solution A into young adult rats, as described by Short et al. [11], produced an increase in the DNA synthetic activity starting at 16 h and peaking at 24 h after the initiation of the infusion (data not shown). Fig. 1 shows that the sialic acid bound to the sinusoidal plasma membrane was decreased after the infusion of solution A. This diminution started at 6 h and the sialic acid values remained decreased at least until 20 h. Minimum amount of sialic acid was observed at 10 h, representing a 32% decrease of the values measured in control animals.

In order to know which component of solution A was responsible for the reduction of sialic acid in the hepatocyte plasma membrane, we measured the content of sialic acid in the sinusoidal membranes at 10 h after the infusion of different mixtures of three components of solution A. As shown in Table I, when rats were infused with a solution containing AGH no decrease in sialic acid was observed; whereas the rats injected with solutions containing TGH, TAG or TAH a 25% decrease was observed in each case. These results strongly indicated that triiodothyronine was the

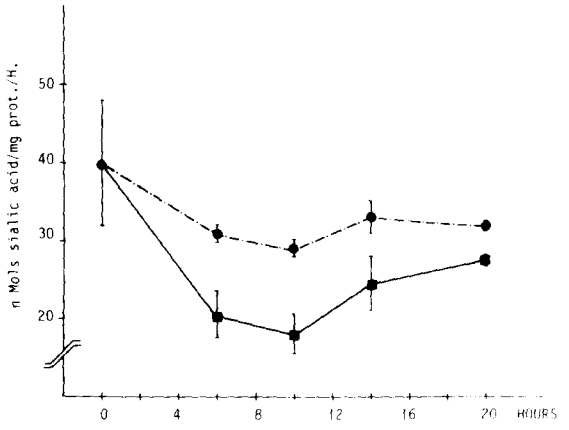

Fig. 1. Content of sialic acid bound to sinusoidal plasma membrane at different times after intravenous injection of TAGH solution. (■) TAGH solution injected rats. (●) Saline injected rats. Each value represents the mean ± S.D. of more than four experiments.

TABLE I

EFFECT OF INFUSION OF DIFFERENT MIXTURES OF THREE COMPONENTS OF TAGH SOLUTION ON SIALIC ACID BOUND TO THE SINUSOIDAL DOMAIN OF HEPATOCYTE PLASMA MEMBRANE

Sialic acid was measured in plasma membrane fraction from rats killed 10 h after the starting of infusion. T, triiodothyronine; A, amino acids; G, glucagon and H, heparin. Each value represents the mean ± S.D. *n*, number of experiments. The evaluation of different treatments has been made using an analysis of variance, in which the means of different groups were significantly different. With the aim to test among which groups were the differences, we have used the ortogonal contrast test [18]. Results obtained from this test showed that: there were no differences between saline solution and AGH solution and also that AGH solution was different to TGH, TAG, TAH and TAGH solutions with $P \leq 0.05$ in all cases.

Solution injected	Sialic acid (nmol/mg protein)	*n*	%
Saline	29 ± 4	8	100
TAGH	18 ± 2	4	68
TGH	21 ± 2	3	75
TAG	22 ± 1	3	76
TAH	22 ± 2	4	76
AGH	26 ± 3	6	93

most relevant component, in the infusion solutions, involved in the reduction of sialic acid bound to the plasma membrane. Furthermore, to determine whether a relationship between DNA synthetic activity and the reduced levels of sialic acid at the plasma membrane can be establised, the incorporation of [^{3}H]thymidine into DNA after the infusion of the different mixtures of three different components of solution A was studied. DNA synthetic activity was measured at 24 h after the infusion (when the maximum of DNA synthesis is accomplished). As shown in Table II, when rats were infused with AGH solution the DNA synthetic activity was similar to control animals. But when animals were infused with a solutions containing TGH, TAG or TAH a significant increase in DNA synthesis was observed. Therefore, the results indicated that only when triiodothyronine was incorporated in the mixture an increased DNA replication was observed. These results correlate very well with those shown in Table I, suggesting that a loss of sialic acid in the plasma membrane can be a process associated to hepatocyte proliferation.

It is becoming clear that the binding of plasma membrane receptors to the components of extracellular matrix is involved in the triggering of cell proliferation and differentiation [15]. Since the sialic acid is a terminal sugar present in the plasma membrane glycoproteins and gangliosides it is possible that changes in the concentration of this carbohydrate can produce alterations in the affinity of the plasma membrane receptors for their ligands in the extracellular matrix and consequently it can be related to the recognition, adhesion and differentiation. We previously reported a decrease in plasma membrane sialic acid of liver cells proliferatively activated by a partial hepatectomy [6] and in this paper a similar alteration has been shown in the liver cells activated by the infusion of solution A. Thus, we conclude that a correlation exist between DNA synthesis and the loss of sialic acid, on the basis of the mixtures infused; and that triiodothyronine critical component of the mixture with regard to the increased DNA synthesis as well as for the loss of sialic acid in the plasma membrane. The results suggest that a diminution of sialic acid in the sinusoidal plasma membrane is a pre-replicative step associated to cell proliferation.

It is not known by which mechanism(s) the T_3 can induce the loss of sialic acid in the plasma membrane as well as the specificity of this process, but since UDP-*N*-acetyl-D-glucosamine 2′-epimerase was inhibited by calmodulin [7] and calmodulin is increased during the pre-replicative phase after proliferative activation [9,10] it could be interesting to test the possibility that T_3 can act by increasing the cytosolic levels of calmodulin.

TABLE II

EFFECT OF THE INFUSION OF DIFFERENT MIXTURES OF THREE COMPONENTS OF TAGH SOLUTION ON DNA SYNTHETIC ACTIVITY

DNA synthetic activity was measured at 24 h after the infusion of these solutions. T, triiodothyronine; A, amino acids; G, glucagon and H, heparin. [^{3}H]Thymidine (0.5 μCi/g body weight) was injected intraperitoneally 1 h before the animals were killed. Each value represents the mean ± S.D. *n*, number of experiments. The statistical analysis of the results was done as described in Table I and gave the same conclusions.

Solution injected	DNA synthetic activity (cpm/g of liver)	*n*	%
Saline	22074 ± 4204	7	100
TAGH	131031 ± 37491	7	594
TGH	65823 ± 13554	4	298
TAG	37887 ± 3240	4	172
TAH	38921 ± 5699	4	176
AGH	20590 ± 3121	4	93

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