Effects of Unconjugated Bilirubin on Bilirubin-UDP-Glucuronyl Transferase Activity in Liver of Newborn Rats

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Extract

The bilirubin-UDP-glucuronyl transferase (BGT) activity in liver of newborn rats was measured to determine if unconjugated bilirubin could be the trigger substance of glucuronyl transferase activity. Comparison was made among normal rats, newborn rats, and rats of mothers that had been given varying doses of unconjugated bilirubin during pregnancy.

Three groups of pregnant Wistar rats were used for the experiments. Group I received bilirubin by intravenous injection once a day during the last five days of pregnancy. Group II received a suspension of bilirubin by intraperitoneal injection during the sixteenth to twenty-first day of pregnancy. Group III served as controls.

Activity was determined by a modification of the method of LATHE and WALKER [14]. Total bilirubin was determined in the incubates according to the method of FOG and BAKKEN [9], while conjugated and unconjugated bilirubin were determined by methods described by FOG and BAKKEN [10].

BGT activity was measured as bilirubin conjugated per gram wet weight liver per hour (table I). Normal newborn rats were unable to conjugate bilirubin at birth, while newborn rats of mothers that had been injected with 150 mg bilirubin for more than 24 hours showed BGT activity at birth. Newborn rats of mothers that had received 150 mg bilirubin less than 24 hours before delivery showed no BGT activity in liver at birth, but the enzyme was activated earlier after birth in these rats than it was in normal rats.

The results show that unconjugated bilirubin triggers UDP-glucuronyl transferase and thus functions as an activator for excretion of bilirubin.

Speculation

The finding that unconjugated bilirubin functions as a trigger for bilirubin-UDP-glucuronyl transferase activity does not explain the molecular mechanism that leads to increased enzyme activity. Further studies are needed to show whether the increased level of activity after birth is due to a new production of ribonucleic acid (RNA), to activation of a protein already present, or to inactivation or withdrawal of an inhibitor. The possible relation between the level of bilirubin in the serum and the conjugating capacity in the newborn should also be studied.

Introduction

Normally, bilirubin-UDP-glucuronyl transferase (BGT) is inactive in the liver of the fetus and the newborn infant [3, 14]. The delayed activation of BGT, however, could be due to inhibitors *in utero* [3, 17], although conjugated bilirubin has been observed in serum at birth [1, 8, 11, 19]. It is unlikely that estrogen inhibitors regulate the activity of BGT at birth, since the concentration of the inhibitors is constant.

That bilirubin alone could trigger UDP-glucuronyl transferase activity is supported by the finding that conjugated bilirubin is present at birth in infants with erythroblastosis fetalis. These circumstances suggest that these infants are able to conjugate earlier than normal newborn infants. The excretory capacity for bilirubin is unexplained. The intrauterine hyperbilirubinemia probably acts as the trigger substance for BGT.

In the present study, a comparison was made between the activity of BGT in the liver of normal rats, newborn rats, and rats loaded with varying doses of unconjugated bilirubin during intrauterine life.

Materials and Methods

Pregnant, six-month-old Wistar rats weighing between 200 and 350 g and fed a Pellet diet were used for the experiments. Rats loaded with bilirubin [22] and normal rats were kept mixed.

Group I

During the last five days of pregnancy an 0.5 ml intravenous injection of 200 mg bilirubin in 0.4 % Na_2CO_3 (anhydr.) at pH 10.9–11.0 was given once a day. The solution had been filtered and kept in the dark until used.

Group II

During the sixteenth to twenty-first day of pregnancy, 3 ml of a bilirubin suspension (10 to 50 mg bilirubin per ml in a mixture of equal volumes of 0.4 % Na_2CO_3 [anhydr.] and 5 mM EDTA [etylendiaminetetracetate] pH 10.2–10.4) was injected intraperitoneally.

Group III

Control animals were given 3 ml of the mixture of Na_2CO_3 and EDTA, with bilirubin omitted.

Preparation of the Tissue and Determination of Enzyme Activity

Following decapitation, the livers were removed immediately and placed in the homogenizing medium, 0.154 M KCl containing $3.2 \cdot 10^{-4}$ M KHCO_3 [5] at $+4^\circ$. Homogenates were prepared by grinding one gram of a mixture of liver from offspring of one mother in 9 ml homogenizing medium in an ice bath. The livers from either two or three newborn rats were mixed, depending on the weight. If less than one gram of liver was used, the volume of the homogenizing medium was reduced accordingly. The mixture was homogenized in a Potter-Elvehjem homogenizer for ten minutes at a constant speed of 80 rpm. Incubation was performed as described by LATHÉ and WALKER [14], with the exception that the bilirubin solution was dissolved in 10 ml of a mixture of equal volumes of 0.4 % Na_2CO_3 (anhydr.) and 5 mM EDTA. The pH was adjusted to 11 by adding dropwise 0.1 N NaOH. This bilirubin solution was filtered and kept in the dark until use. Activity was reported as μg of bilirubin conjugated/g wet weight of liver/h ($\mu\text{g/gmw/h}$).

Total bilirubin in the incubates was determined by the method of FOG and BAKKEN [9] and conjugated and unconjugated bilirubin were determined by direct spectrophotometry [10], inasmuch as the azo-methods for bilirubin determination in *in vitro* determination of glucuronide formation were not recommended [2]. Total bilirubin in sera from pregnant rats was determined by the method of JENDRASSIK and GROF [13] as modified by FOG [7]. Bilirubin in serum of newborn rats was not measured.

Results

Newborn and one-day-old normal rats showed no hepatic BGT-activity. At two days of age, activity varied from 0 to 60 $\mu\text{g/gmw/h}$. Subsequently, the activity increased to 225 $\mu\text{g/gmw/h}$ and was stabilized at about 275 $\mu\text{g/gmw/h}$ after fourteen days.

Total bilirubin in sera of untreated pregnant rats varied from 0.2 to 0.5 mg bilirubin per 100 ml.

Group I

BGT-activity in the newborn rats of mothers that had been loaded with 200 mg of bilirubin intravenously once a day during the last five days of pregnancy did not differ from that in normal newborn rats.

Total bilirubin in the sera of these pregnant rats did not exceed 0.6 mg/100 ml.

Group II

BGT-activity at birth in rats whose mothers had received 150 mg of bilirubin more than 24 hours before

Table I. Bilirubin-UDP-glucuronyl transferase activity in liver of rats

Days after birth	μg bilirubin conjugated/g wet weight liver/h											
	0	1	2	3	4	5	8	9	14	21	28	Adults
No load	0	0	0	90	144	180	150	84	132	276	288	248
	0	0	30	100	160	182	180	198	380	384	270	260
	0	0	60			210	265	240	368	276		280
	0	0				264	278	180	180	372		292
						216	210	165	320			316
Offsprings from 17 mothers						230	216	180				330
							180					552
Bilirubin given less than 24 hours before birth	0	60	120	178	285							
	0	110	120	136	310							
	0	110		144								
Offsprings from 9 mothers	0			180								
Bilirubin given more than 24 hours before birth	0	24	80	72	56	252	450	486	225	195		
	12	46	140	144	90	252	450	504	265	390		
	25	86	140	180	160				282			
Offsprings from 21 mothers	35	102	178	240	172				300			
	37				200				345			
	60				238							
	62											
	70											
	75											

birth varied from 0 to 78 $\mu\text{g/gmw/h}$. After 24 hours, values ranged from 25 to 100 $\mu\text{g/gmw/h}$, with an average value of 65 $\mu\text{g/gmm/h}$. The maximal value, 335 $\mu\text{g/gmw/h}$, was reached at eight to ten days of age. Total bilirubin in the sera of these pregnant rats varied from 1.3 to 2.6 mg/100 ml.

BGT activity in offspring of the control rats did not differ from that found in normal newborn rats.

Newborn rats whose mothers were loaded with bilirubin less than 24 hours prior to birth showed no activity in liver at the time of birth; activity was detectable on the second day of life (0–110 $\mu\text{g/gmw/h}$). After four days of life, activity was stabilized at about 300 $\mu\text{g/gmw/h}$.

BGT activity of newborn rats whose mothers received 50 and 100 mg of bilirubin intraperitoneally one to five days before birth did not differ from that found in the liver of normal newborn rats.

Total bilirubin in the serum of these pregnant rats varied from 0.6 to 1.2 mg/100 ml. There was no correlation between BGT activity in the newborn and the level of bilirubin in the mothers when the level was higher than 1.3 mg/100 ml.

Discussion

The present study shows a possible mechanism for the activation of the bilirubin conjugating system. Excretion of bilirubin was not studied.

The results show that BGT is inactive in normal newborn rats. Activity of this enzyme is measurable a few days after birth; however, newborn rats of mothers with hyperbilirubinemia conjugated bilirubin at birth. Although the delayed activation of BGT in the normal newborn has been reported to be caused by estrogens [12, 17], we do not believe that these inhibitors are important, since BGT is active *in utero* in infants with erythroblastosis fetalis [1]. The level of estrogens is no lower in these infants as compared to normal subjects. In addition, estrogen inhibitors of BGT affect only intact cells and not the enzyme in homogenates [15]. This fact must mean that the estrogens inhibit the transport of bilirubin through the cell wall and not the enzyme activity *per se*. Therefore, homogenates were used in this study to eliminate any error resulting from a failure of bilirubin to penetrate intact cell wall. Activity of BGT is related to the concentration of un-

conjugated bilirubin, according to SCHMID *et al.* [19]. These authors were unsuccessful in an attempt to stimulate glucuronidation of o-aminophenol by intraperitoneal loading with sodium antralinate. Later, LESTER and SCHMID and others [6, 16, 21] showed the importance of using bilirubin as a substrate for studies of BGT.

Table I shows the levels of BGT activity in the liver of normal rats, newborn rats, and rats of mothers loaded with varying amounts of bilirubin. Newborn rats of mothers loaded with an intraperitoneal injection of 150 mg of bilirubin more than 24 hours before delivery showed BGT activity at birth. The unconjugated bilirubin served as a depot from which bilirubin escaped at such a rate that the liver of the pregnant rat was unable to conjugate all of it. The level of bilirubin in serum increased to 1.3 to 2.6 mg/100 ml, four times greater than that in the control rats. Since unconjugated bilirubin in serum can pass through the placenta [18] there was a parallel increase in the level of bilirubin in serum in the rat fetus. This increase in the fetus acted as a trigger for BGT activity.

Levels of bilirubin were not measured in serum of newborn rats used in these studies. The experiments were designed to identify a difference between the BGT activity in the normal newborn rat and the newborn rat whose mother was loaded with bilirubin. A difference was noted even in the newborn rats whose mothers were loaded less than 24 hours before delivery. These rats did not show any BGT activity at birth, but their livers were able to conjugate bilirubin earlier after birth than were those from normal rats. These results indicate that the enzyme activating process had started *in utero* even in these cases, but that 24 hours are too short a time for the process of stimulation to be completed.

These studies, therefore, show that unconjugated bilirubin appears to act as a trigger for glucuronyl transferase activity.

Summary

Pregnant Wistar rats were loaded with bilirubin during the last days of pregnancy. The newborn rats of mothers that had been given 150 mg of bilirubin by intraperitoneal injection more than 24 hours before birth were able to conjugate bilirubin at birth. However, normal newborn rats did not show this ability until about two days after birth. Newborn rats of mothers loaded with 150 mg bilirubin less than 24 hours before birth were unable to conjugate bilirubin at birth, but developed this ability earlier in life than did normal newborn rats.

It appears, therefore, that unconjugated bilirubin

is a trigger substance for bilirubin-UDP-glucuronyl transferase activity in the newborn period.

References and Notes

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