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SUPPRESSION OF GLYCOGEN STORAGE WITH BETAMETHASONE 17,21-DIPROPIONATE IN FETAL RAT LIVER—I. EFFECT ON METABOLITES OF THE GLUCOGENIC PATHWAY AND GLYCOGEN SYNTHASE ACTIVITY

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Abstract—Betamethasone 17,21-dipropionate, a potent synthetic glucocorticoid, showed dose-responsive suppression of glycogen accumulation during late pregnancy in fetal rat liver when it was administered on day 19 of gestation. Complete suppression occurred at a dose of 100 μg/fetus. A similar result was obtained with decapitated fetuses. The change in the levels of metabolites of the glycogenic pathway was examined 5, 10 and 24 hr after injection of 25 or 100 μg of the steroid/fetus. Blockade of glycogen accumulation was observed at 10 hr, as the age-dependent increase in glycogen in control fetuses was detected at that time. UDP-glucose concentration increased at 5 hr and reached a maximal level at 10 hr with doses of 25 and 100 μg/fetus. Glucose-1-P, which was expected to increase with stimulated glycogenolysis, increased after 5 hr at a dose of 25 μg/fetus but not at 100 μg/fetus. Glucose-6-P gradually decreased from day 19 to 21 in control fetuses but this age-dependent decrease was blocked by the steroid. Glucose increased after 5 hr and remained at the same level thereafter. The steroid also decreased glycogen synthase (EC 2.4.1.11) *a* activity. These results indicate that betamethasone 17, 21-dipropionate suppresses glycogen accumulation in fetal rat liver, and inhibition between UDP-glucose and glycogen plays an important role in this action.

Administration of glucocorticoids generally has induced atrophy of the adrenals in adult and fetal animals [1–3], but betamethasone 17, 21-dipropionate, a potent synthetic glucocorticoid [4], induced hypertrophy of the adrenals in rat fetuses [5]. These findings having raised the question of whether this steroid acts on other target organs as a glucocorticoid. Because glycogen accumulation in fetal rat liver during late pregnancy is stimulated by treatment with glucocorticoid [6–8], we have examined the effect of betamethasone 17, 21-dipropionate on glycogen accumulation in fetal rat liver. This steroid markedly suppressed glycogen accumulation during late pregnancy, with impairment of the step between UDP-glucose and glycogen being the cause of this. This steroid also decreased the activity of glycogen synthase.

MATERIALS AND METHODS

Animals. Adult female JCL Sprague–Dawley strain (CLEA Japan, Inc, Tokyo) rats were mated over a 12-hr period and those which had sperm present in their vaginal smears were considered to be at day 0 of gestation. On day 19 of gestation, the mothers were laparotomized under ether anesthesia and the fetuses were injected s.c. through the uterine walls with a suspension of steroid in 0.5% acacia gum solution. Decapitation *in utero* of fetuses was performed on day 19 of gestation by the method of Milkovic and Milkovic [9]. The pregnant rats were killed by a blow to the head and then the fetuses were removed immediately and their livers were excised. The livers were frozen immediately in liquid nitrogen and stored on dry ice.

Assay of metabolites. The procedures were based on published fluorometric methods [10]. Tissue was homogenized in ice-cold 1 N $HClO_4$ in a final volume of 3.0 ml and the homogenate was centrifuged. The samples for determination of glycogen [11] and P_i [12] were obtained from the supernatant fraction. For determination of the other metabolites, the supernatant fraction was neutralized with 1 N $KHCO_3$ and treated with Florisil to remove most of the fluorescent materials [13].

Assay of glycogen synthase. Glycogen synthase activity was measured by the method of Leloir and Goldemberg [14]. A 10% homogenate was prepared in ice-cold 0.4 M sucrose–5 mM EDTA solution (pH 8.0). The assay mixture, in a final volume of 100 μl, contained 0.25 M glycine–5 mM EDTA buffer (pH 8.5), 6 mM cysteine, 0.8 mg glycogen, 5 mM UDP-glucose and 10 μl homogenate. The total ($a + b$) activity was determined in the presence of 10 mM glucose-6-P and that of synthase *a* in the presence of 10 mM Na_2SO_4 [15]. Enzyme activity was expressed as μmoles UDP formed/min/g of liver.

Assay of phosphorylase. Phosphorylase (EC 2.4.1.1) activity was measured by the method of Schwartz and Rall [16]. The liver was homogenized in ice-cold 75 mM NaF–5 mM EDTA (pH 6.7). The reaction mixture (pH 6.1), in a final volume of 0.65 ml, contained 11.2 mM glucose-1-P, 0.1 ml NaF, 3.4 mM EDTA, 0.8 mg glycogen, 2.5 mM 5′-AMP and 0.1 ml homogenate. Enzyme activity was expressed as μmoles P_i released/min/g of liver. The total ($a + b$) activity was determined by converting the *b* form into the *a* form by treatment with purified phosphorylase

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kinase (EC 2.7.1.38). Phosphorylase kinase was prepared from rabbit muscle by the method of Krebs *et al.* [17] and activated before use [17].

Chemicals. Glycogen, glucose-6-P, glucose-1-P, NAD, NADH, ATP, phosphoenolpytuvate, and lactate dehydrogenase (EC 1.1.1.27) (type III) were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A., UDP-glucose, NADP, cyclic AMP and 5′-AMP from Kojin Co., Japan, and glucose-6-phosphate dehydrogenase (EC 1.1.1.49), phosphoglucomutase (EC 2.7.5.1), hexokinase (EC 2.7.1.1), UDP-glucose dehydrogenase (EC 1.1.1.22), pyruvate kinase (EC 2.7.1.40) and myokinase (EC 2.7.4.3) from Boehringer-Mannheim GmbH, Mannheim, West Germany. Betamethasone 17,21-dipropionate was obtained from the Shering Corp., Bloomfield, NJ, U.S.A.

RESULTS

Effects on glycogen concentration. Glycogen concentration in fetal rat liver increases sharply after 19 days of gestation [6–8, 18, 19]. As shown in Fig. 1, betamethasone 17,21-dipropionate completely suppressed the age-dependent increase in liver glycogen at a dose of 100 μg/fetus; the glycogen level remained the same as on day 19. The suppression of glycogen accumulation was statistically significant at doses above 25 μg of steroid/fetus.

The steroid suppressed glycogen accumulation in decapitated fetuses as markedly as in intact fetuses (Table 1). The results suggest that the effect of this steroid on glycogen accumulation in the liver is not mediated by the central nervous system.

Effects on metabolites of the glycogenic pathway. Table 2 shows the effects of betamethasone 17,21-dipropionate on metabolites of the glycogenic pathway. Liver glycogen accumulated rapidly from day 19 to 20 of gestation in control fetuses. Blockade of this accumulation occurred from 10 hr after administration of the steroid. Increase in UDP-glucose was detected 5 hr after administration of the steroid and reached a maximum 10 hr after administration. In control fetuses, the concentrations of glucose-1-P and glucose-6-P gradually decreased from day 19 to 21. Administration of 25 μg of the steroid/fetus caused an increase in glucose-1-P by 5 hr and blocked the age-dependent decrease in glucose-1-P from day 19 to 20. The effect of 100 μg of the steroid/fetus on glucose-1-P concentration was less than the effect of 25 μg/fetus and the increase was observed only 24 hr after administration. The age-dependent decrease in glucose-6-P was inhibited at doses of 25 and 100 μg/fetus. These results indicate that administration of betamethasone 17,21-dipropionate impairs mainly the glucosyl transfer step from UDP-glucose to glycogen.

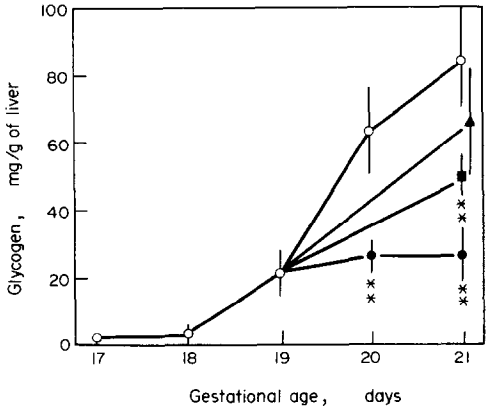

Fig. 1. Effect of betamethasone 17,21-dipropionate on glycogen concentrations in fetal rat liver. The fetuses were injected s.c. *in utero* with various doses of this steroid or 20 μl vehicle on day 19 of gestation. Vehicle, ○; 10 μg steroid/fetus, ▲; 25 μg steroid/fetus, ■; 100 μg steroid/fetus, ●. Values are means ± S.D. of six to twelve fetuses from two litters. A double asterisk indicates a significant difference ($P < 0.01$) from the corresponding control (vehicle).

Table 1. Effect of betamethasone 17, 21-dipropionate on glycogen concentration in the livers of decapitated rat fetuses*

Treatment	Glycogen (mg/g of liver)
Vehicle (20 μl of 0.5% acacia)	59 ± 8
Betamethasone 17, 21-dipropionate (100 μg/fetus)	7 ± 4†

* Decapitation and s.c injection of the steroid *in utero* were carried out on day 19 of gestation. The fetuses were delivered by caesarean section on day 21. Values are means ± S.D. of eight to nine fetuses from two litters.

† Significantly different ($P < 0.01$) from vehicle control.

The glucose concentration increased 5 hr after administration of betamethasone 17, 21-dipropionate and remained at the increased level thereafter. This result indicates that the glycogen accumulation suppressed by the steroid is not due to a deficiency of glucose.

Effect of glycogen synthase activity. Glycogen synthase catalyzes the step between UDP-glucose and glycogen. Betamethasone 17,21-dipropionate decreased the activity of glycogen synthase *a* at doses of 25 and 100 μg/fetus 10 hr after administration on day 19 of gestation, while it increased the total activity of synthase (Table 3). Twenty-four hr after administration of the steroid on day 19 of gestation, the age-dependent increases in the total ($a + b$) and the *a* activities of glycogen synthase were partially suppressed (Fig. 2). The steroid did not affect the rate of decrease in synthase activity from day 20 to 21. Betamethasone 17, 21-dipropionate did not inhibit glycogen synthase *in vitro*.

Effect on phosphorylase activity. The rate-limiting step of glycogen breakdown in the liver is catalyzed by phosphorylase. The total activity of phosphorylase was not affected by the steroid. The activity level of phosphorylase *a* in the steroid-treated fetuses was higher than the level in vehicle-treated fetuses 24 hr after administration on day 19 of gestation but this difference was not observed after 48 hr (Fig. 3).

DISCUSSION

Glycogen concentration in fetal rat liver increases sharply after 19 days of gestation [6–8, 16, 18, 19] and glucocorticoids are indispensable for this accumulation

Table 2. Effects of betamethasone 17,21-dipropionate on five metabolites in fetal rat liver*

Treatment	Time after injection (hr)	Glycogen†	UDP-glucose‡	G-1-P‡	G-6-P‡	Glucose‡
Vehicle (20 μl of 0.5% acacia)	5	35.0 ± 6.1	0.152 ± 0.011	0.026 ± 0.002	0.218 ± 0.030	1.56 ± 0.19
	10	90.6 ± 16.1	0.173 ± 0.034	0.028 ± 0.006	0.177 ± 0.016	1.71 ± 0.11
	24	320.6 ± 38.3	0.180 ± 0.007	0.022 ± 0.002	0.129 ± 0.014	2.07 ± 0.20
	48	361.7 ± 67.2	0.136 ± 0.013	0.015 ± 0.008	0.090 ± 0.013	2.20 ± 0.16
Betamethasone 17, 21-dipropionate (25 μg/fetus)	5	44.4 ± 14.4	0.179 ± 0.018§	0.032 ± 0.005§	0.231 ± 0.044	2.25 ± 0.74‖
	10	28.3 ± 5.6§	0.203 ± 0.012‖	0.035 ± 0.004§	0.218 ± 0.022§	2.19 ± 0.33§
	24	86.7 ± 17.2§	0.191 ± 0.018	0.033 ± 0.005§	0.235 ± 0.042§	2.30 ± 0.20‖
Betamethasone 17, 21-dipropionate (100 μg/fetus)	5	77.8 ± 16.1	0.197 ± 0.024§	0.028 ± 0.004	0.250 ± 0.053	2.37 ± 0.22§
	10	37.8 ± 10.6§	0.224 ± 0.011§	0.029 ± 0.003	0.224 ± 0.044§	2.22 ± 0.17§
	24	70.6 ± 30.0§	0.160 ± 0.018§	0.031 ± 0.004§	0.218 ± 0.038§	2.41 ± 0.26§
	48	37.8 ± 21.0§	0.129 ± 0.018	0.018 ± 0.005	0.179 ± 0.028§	2.54 ± 0.35‖

* The steroid was injected s.c. *in utero* at 9:00 a.m. on day 19 of gestation. Values are means ± S.D. of ten fetuses from two litters.

† Values are μmoles glucose/g of liver.

‡ Values are μmoles/g of liver.

§ Significantly different ($P < 0.01$) from vehicle control.

‖ Significantly different ($P < 0.05$) from vehicle control.

of glycogen [6, 20]. Administration of betamethasone 17,21-dipropionate, a potent synthetic glucocorticoid, to intact or decapitated rat fetuses on day 19 of gestation markedly suppressed the glycogen accumulation. Hasegawa *et al.* [5] found that this synthetic steroid induces hypertrophy of the adrenals by acting on the hypothalamo-pituitary system in rat fetuses. This shows that betamethasone 17, 21-dipropionate has an effect not only on the central nervous system but also on the liver in rat fetuses.

Hornbrook *et al.* [13] reported that adrenalectomy impairs the step between UDP-glucose and glycogen and causes almost complete conversion of glycogen synthase into the *b* form, while cortisol administration reverses these changes. Betamethasone 17,21-dipropionate suppressed the step between UDP-glucose and glycogen and the glycogen synthase activity; these findings are similar to phenomena induced by adrenalectomy. Therefore, this steroid probably suppressed the glycogen accumulation by inhibiting the action of endogenous corticosterone on glycogenesis. This conclusion is supported by the observation that betamethasone 17,21-dipropionate suppressed the glycogenic action of cortisol in fetal rat liver explants [21].

Increase in glycogen synthase during late pregnancy in fetal rat liver is also dependent on glucocorticoids [22]. The decrease in fetal synthase activity 24 and 48 hr after administration of betamethasone 17,21-dipropionate is interpreted as an inhibition of the action of corticosterone on induction of glycogen synthase. The increase in total synthase activity 10 hr after administration of the steroid is not understood.

In fetal rat liver explants, insulin stimulates the conversion of glycogen synthase into the *a* form [16, 23] and its concentration increases in the plasma of rat fetuses from day 18.5 to 20.5 of gestation [24]. Because betamethasone 17,21-dipropionate partially suppressed the glycogenic action of insulin in fetal rat liver explants [21], the inhibitory effect of the steroid on the action of insulin also probably plays an important role in the suppression by the steroid of the age-dependent increase in glycogen synthase *a* activity.

It has been reported that the regulation of glycogen synthesis may be the major factor in the hormonal

Table 3. Effect of betamethasone 17,21-dipropionate on glycogen synthase activity in fetal rat liver*

Treatment	Glycogen synthase (μmoles/min/g of liver)	
	a + *b*	*a*
Vehicle (20 μl of 0.5% acacia)	1.94 ± 0.32	0.61 ± 0.26
Betamethasone 17, 21-dipropionate (25 μg/fetus)	2.19 ± 0.19†	0.42 ± 0.11†
(100 μg/fetus)	2.30 ± 0.25†	0.35 ± 0.09‡

* Fetuses were injected s.c. *in utero* with the steroid at 9:00 a.m. on day 19 of gestation and delivered 10 hr after injection by caesarean section. Values are means ± S.D. of ten fetuses from two litters.

† Significantly different ($P < 0.05$) from vehicle control.

‡ Significantly different ($P < 0.01$) from vehicle control.

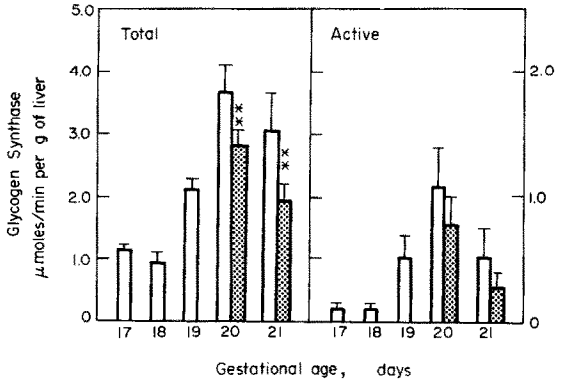

Fig. 2. Effect of betamethasone 17,21-dipropionate on glycogen synthase activity in fetal rat liver. The fetuses were injected s.c. *in utero* with 100 μg of the steroid (▨)/fetus or 20 μl vehicle on day 19 of gestation. Values are means ± S.D. of eight fetuses from two litters. A double asterisk indicates a difference ($P < 0.01$) from the corresponding control.

control of glycogen metabolism in fetal rat liver [16, 19] and Watts and Gain [25] have suggested that there could be a continual cycling of glucose through glycogen under basal conditions. According to this view, if betamethasone 17,21-dipropionate does not stimulate the glycogenolytic process but suppresses glycogen synthesis, glycogen breakdown may exceed its synthesis, which may result in the increase of glucose, glucose-6-P and glucose-1-P. Since the synthetic steroid significantly increased phosphorylase *a* activity 24 hr after its administration at 100 μg/fetus, it may stimulate glycogenolysis. However, the stimulated glycogenolysis may not be the major cause of suppressed glycogen accumulation, as the increase of phosphorylase *a* was not observed 48 hr after administration of betamethasone 17,21-dipropionate in spite of the completely abolished glycogen accumulation at that time.

In conclusion, the impaired glycogen storage by betamethasone 17,21-dipropionate was mainly due to suppressed glycogen synthesis rather than stimulated glycogen breakdown, though the latter may play a minor role. Because the steroid stimulated endogenous corticosterone production (Y. Hasegawa and Y. Mizushima, unpublished observations), inhibition of the action of endogenous corticosterone by betamethasone 17,21-dipropionate, probably at the receptor level, may be a major cause of impaired glycogenesis, although suppression of the action of insulin may also be a cause.

The activities of glycogen synthase and phosphorylase are modulated by glucose-6-P, UDP-glucose, P_i and adenine nucleotides [26]. The concentrations of these effectors were significantly changed by betamethasone 17,21-dipropionate (data of P_i and adenine nucleotides were not shown), but the small absolute changes of effector levels minimize the importance of the modulation of enzyme activities in regard to the suppression of glycogen accumulation.

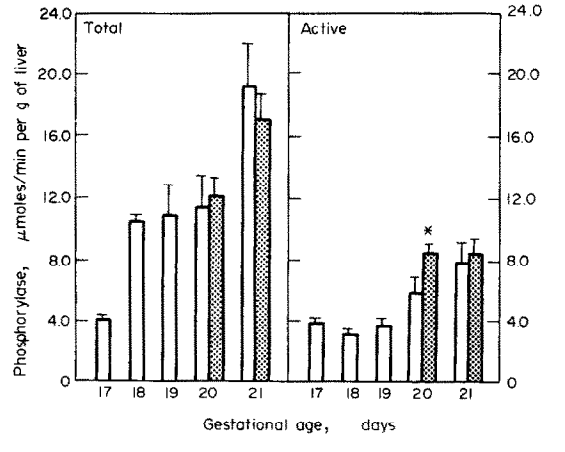

Fig. 3. Effect of betamethasone 17,21-dipropionate on phosphorylase activity in fetal rat liver. The fetuses were injected s.c. *in utero* with 100 μg of the steroid (▨)/fetus or 20 μl vehicle on day 19 of gestation. Values are means ± S.D. of eight fetuses from two litters. An asterisk indicates a significant difference ($P < 0.05$) from the corresponding control.

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