Biochemical Pharmacology, Vol. 28, pp. 741–745.

0006-2952/79/0315-0741 $02.00/0

SUPPRESSION OF GLYCOGEN STORAGE WITH BETAMETHASONE 17,21-DIPROPIONATE IN FETAL RAT LIVER—II. INHIBITION OF GLYCOGENIC ACTION OF CORTISOL AND INSULIN IN THE LIVER IN ORGAN CULTURE

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(*Received* 21 *March* 1978; *accepted* 14 *August* 1978)

Abstract—The effect of betamethasone 17,21-dipropionate, a potent synthetic glucocorticoid, on the glycogenic actions of cortisol and insulin was examined with fetal rat liver in organ culture. This steroid itself had no glycogenic action, unlike betamethasone or cortisol. To the contrary, at 10^{-7} M, it suppressed completely the glycogenic action of cortisol (10^{-7} M) and was suppressive even at 10^{-9} M. Cortisol-induced glycogen accumulation was interrupted upon addition of betamethasone 17,21-dipropionate. The glycogenic action of cortisol was exhibited in explants preincubated with betamethasone 17,21-dipropionate upon replacement of the medium with one containing cortisol only. Betamethasone 17,21-dipropionate partially suppressed insulin-induced glycogen accumulation when added simultaneously with insulin (4.0 μg/ml), but could not interrupt such glycogen accumulation already in progress.

Unlike the natural glucocorticoids, betamethasone 17,21-dipropionate, a potent synthetic glucocorticoid [1], suppresses glycogen accumulation during late pregnancy in fetal rat liver *in utero* [2]. In order to clarify the mechanism of action, studies *in vitro* were performed. Glucocorticoids and insulin are glycogenic hormones and are effective in fetal rat liver in organ culture [3, 4,]. The present paper shows the effect of betamethasone 17,21-dipropionate on cortisol- and insulin-induced glycogen accumulation in fetal rat liver in organ culture.

MATERIALS AND METHODS

Organ culture. Livers were removed under sterile conditions from fetal rats (JCL Sprague–Dawley strain, CLEA Japan Inc.) on day 19 of gestation. The organ culture was carried out for the most part by the method of Wicks [5]. Livers were cut into about 1-mm cubes, and the fragments were placed on stainless steel grids in a tissue culture dish. About 5 ml of Eagle's minimum essential medium with Earle's balanced salt solution (pH 7.4), containing twice the usual amounts of glucose, was added to just wet the lower surface of the explants. The dishes were placed in a humidified incubator at 37° with a circulating gas phase of 95% air–5% CO_2. The medium was replaced once daily. The mean wet weight of tissue per dish was about 20 mg after 42 hr of incubation.

Assay of glycogen. The explants were homogenized in 0.4 N $HClO_4$ and the homogenates were centrifuged. Glycogen was purified from the supernatant fraction by the method of van Handel [6] and then measured by the anthrone method.

Chemicals. Eagle's MEM and Earle's BSS were obtained from Nissui Seiyaku Co. Ltd., Tokyo, Japan. Cortisol acetate was obtained from Nakarai Chemical Ltd., Kyoko, Japan, betamethasone from the Sigma Chemical Co., St. Louis, MO, U.S.A., and betamethasone 17,21-dipropionate from the Shering Corp., Bloomfield, NJ, U.S.A. Insulin (porcine) was a kind gift of the Eli Lilly Research Laboratories, Indianapolis, IN, U.S.A. Betamethasone 17α-monopropionate was a generous gift of Dr. Y. Tomita in our laboratories.

RESULTS

Effect on cortisol-induced glycogen accumulation. Figure 1 shows the course of cortisol-induced glycogen accumulation in relation to the culture period. When cortisol was added after 18 hr of preincubation in basal medium, the glycogen concentration reached a maximum level within 24 hr after addition and this level was maintained until 66 hr. When cortisol was added after 42 hr of preincubation, the response was a little weaker. Addition of cortisol at the beginning of culture was somewhat inadequate because glycogen levels were more variable and still decreasing.

The optimum concentration of cortisol for glycogen accumulation was 10^{-7} M as shown in Table 1.

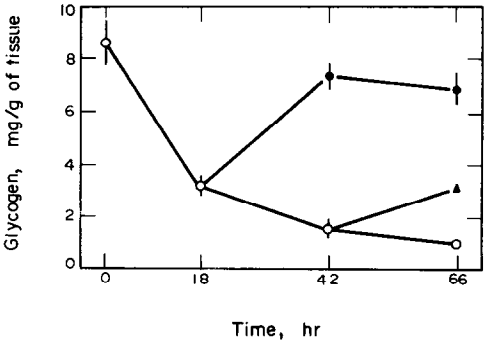

Fig. 1. Time course of cortisol-induced glycogen accumulation. Cortisol acetate (2×10^{-6} M) was added at 18 hr (●) or 42 hr(▲) of culture. Each point represents the mean ± S.D. of explants from five separate dishes.

Table 1. Dose response to cortisol *

Cortisol (M)	Glycogen (mg/g of tissue)
0	1.19 ± 0.11
3×10^{-9}	1.90 ± 0.35
1×10^{-8}	2.83 ± 0.45
3×10^{-8}	3.53 ± 0.50
1×10^{-7}	5.98 ± 0.30
3×10^{-7}	5.31 ± 0.85
1×10^{-6}	4.89 ± 0.61

* Explants were incubated for 18 hr in control medium and then exposed to various doses of cortisol acetate for 24 hr. Each value is the mean ± S. D. of explants from five separate dishes.

Table 2 shows the effect of betamethasone 17,21-dipropionate on glycogen accumulation induced by 10^{-7} M cortisol. The accumulation was completely suppressed with 10^{-7} M betamethasone 17,21-dipropionate and decreased by 57 per cent in the presence of 10^{-9} M. Betamethasone 17,21-dipropionate itself had no glycogenic effect at 10^{-6} and 10^{-7} M.

Betamethasone 17,21-dipropionate interrupted cortisol-induced glycogen accumulation when added after it had started (Fig. 2). Removal of cortisol from the culture medium also interrupted the accumulation, as reported by Plas *et al.* [7] for cultures of fetal rat hepatocytes.

Table 2. Effects of betamethasone 17,21-dipropionate on cortisol-induced glycogen accumulation. *

Addition		
Cortisol (M)	Betamethasone 17,21-dipropionate (M)	Glycogen (mg/g of tissue)
0	0	1.25 ± 0.14
10^{-7}	0	5.08 ± 0.68
10^{-7}	10^{-10}	4.63 ± 0.66
10^{-7}	10^{-9}	3.42 ± 0.55
10^{-7}	10^{-8}	1.76 ± 0.40
10^{-7}	10^{-7}	1.29 ± 0.24
0	10^{-7}	1.10 ± 0.15
0	10^{-6}	0.99 ± 0.07

* Explants were incubated for 18 hr in control medium and then exposed to cortisol acetate and betamethasone 17,21-dipropionate for 24 hr. Each value is the mean ± S. D. of explants from five separate dishes.

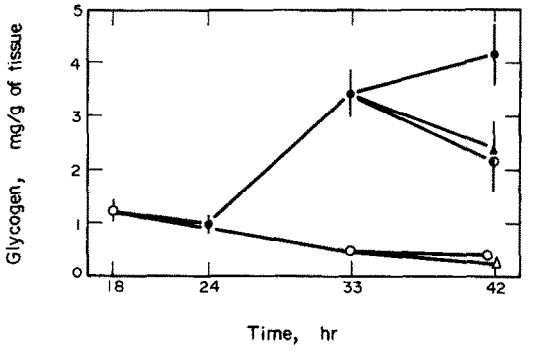

Fig. 2. Addition of betamethasone 17,21-dipropionate during progress of cortisol-induced glycogen accumulation. Explants were exposed to cortisol acetate (10^{-7} M) (●) at 18 hr of culture. At 33 hr of culture, betamethasone 17,21-dipropionate (10^{-7} M) was added (▲, △) or cortisol was removed (◑). Each point represents the mean ± S.D. of explants from five separate dishes.

Table 3 shows the effect of preincubation with betamethasone 17,21-dipropionate on glycogen accumulation induced by cortisol. The explants were preincubated with 10^{-7} M betamethasone 17,21-dipropionate during a culture period of 18–42 hr, then transferred and cultured for a further 24 hr in a medium containing 10^{-7} M cortisol. The glycogen accumulation was about 60 per cent of that of the control.

Glycogenic actions of betamethasone 17α-monopropionate, betamethasone and propionate. Betamethasone 17,21-dipropionate was hydrolyzed to betamethasone via betamethasone 17α-monopropionate in the

Table 3. Effects of preincubation with betamethasone 17,21-dipropionate on cortisol-induced glycogen accumulation *

Addition		Total incubation time (hr)	Glycogen (mg/g of tissue)
18–42 hr	42–66 hr		
None		42	0.78 ± 0.15
None	None	66	0.34 ± 0.15
None	Cortisol	66	2.31 ± 0.47
Betamethasone 17,21-dipropionate		42	0.85 ± 0.11
Betamethasone 17,21-dipropionate	None	66	0.68 ± 0.08
Betamethasone 17,21-dipropionate	Cortisol	66	1.59 ± 0.31

* Explants were incubated with betamethasone 17,21-dipropionate (10^{-7} M) from 18 to 24 hr and then exposed to cortisol acetate (10^{-7} M) for 24 hr. Each value is the mean ± S. D. of explants from five separate dishes.

liver (personal communication from Dr. Y. Tomita in our laboratories). Table 4 shows the glycogenic action of these three compounds and their effects on cortisol-induced glycogen accumulation. Betamethasone showed a glycogenic action equal to that of cortisol.

Table 4. Effects of betamethasone 17α-monopropionate, betamethasone and propionate on cortisol-induced glycogen accumulation *

Addition	Glycogen (mg/g of tissue)
None	1.22 ± 0.25
Cortisol	5.89 ± 1.04
Betamethasone 17α-monopropionate	3.13 ± 0.84
Cortisol + betamethasone 17α-monopropionate	3.96 ± 0.89
Propionate	1.06 ± 0.20
Cortisol + propionate	5.56 ± 0.65
Betamethasone	6.09 ± 1.11

* Explants were incubated for 18 hr in control medium and then exposed to the compounds (10^{-7} M). Values are means ± S. D. of explants from five separate dishes.

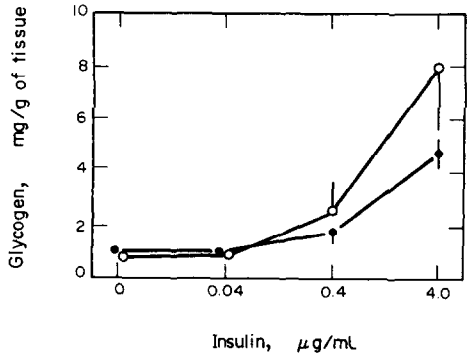

Fig. 3. Effect of betamethasone 17,21-dipropionate on insulin-induced glycogen accumulation. Explants were incubated for 18 hr in control medium, then exposed to insulin (○) or insulin plus betamethasone 17,21-dipropionate (10^{-7} M) (●) for 24 hr. Each point represents the mean ±S.D. of explants from five separate dishes.

Table 5. Effects of betamethasone 17,21-dipropionate on insulin-induced glycogen accumulation *

Addition		
Insulin (μg/ml)	Betamethasone 17,21-dipropionate (M)	Glycogen (mg/g of tissue)
0	0	0.33 ± 0.05
4	0	5.20 ± 1.43
4	10^{-10}	5.52 ± 1.05
4	10^{-9}	4.00 ± 0.68
4	10^{-8}	2.34 ± 1.10
4	10^{-7}	1.82 ± 0.80
4	10^{-6}	1.60 ± 0.50

* Explants were incubated for 18 hr in control medium and then exposed to insulin and betamethasone 17,21-dipropionate for 24 hr. Values are means ± S.D. of explants from five dishes.

Propionate showed neither a glycogenic action nor a suppression of cortisol-induced glycogen accumulation. Betamethasone 17α-monopropionate showed a weaker glycogenic action than cortisol and suppressed the action of cortisol. These results indicate that the suppressive action of betamethasone 17,21-dipropionate on glycogen accumulation in fetal rat liver during late pregnancy is mainly due to the steroid itself.

Effect on insulin-induced glycogen accumulation. Insulin showed glycogenic action above the concentration of 0.4 μg/ml. Betamethasone 17,21-dipropionate suppressed about 50 per cent of the glycogen accumulation caused by 0.4 and 4.0 μg/ml of insulin (Fig. 3). Even at 10^{-6} M, it did not completely suppress the glycogen accumulation induced by 4.0 μg/ml of insulin (Table 5). The suppression of insulin-induced glycogen accumulation was not statistically significant at concentrations below 10^{-9} M. Table 6 shows that preincubation with the steroid did not affect the insulin-induced glycogen accumulation. Betamethasone 17,21-dipropionate also did not interrupt the accumulation, while removal of insulin did (Fig. 4).

Effect on histological appearance. Betamethasone 17,21-dipropionate suppressed the glycogenic actions of cortisol and insulin, and this effect may be a specific

Table 6. Effects of preincubation with betamethasone 17,21-dipropionate on insulin-induced glycogen accumulation *

Addition		Total incubation time (hr)	Glycogen (mg/g of tissue)
18–42 hr	42–66 hr		
None		42	0.45 ± 0.08
None	None	66	0.53 ± 0.15
None	Insulin	66	1.38 ± 0.18
Betamethasone 17,21-dipropionate		42	0.50 ± 0.04
Betamethasone 17,21-dipropionate	None	66	0.47 ± 0.07
Betamethasone 17,21-dipropionate	Insulin	66	1.73 ± 0.39

* Explants were incubated with betamethasone 17,21-dipropionate (10^{-7} M) from 18 to 42 hr and then exposed to insulin (4.0 μg/ml) for 24 hr. Values are means ± S.D. of explants from five separate dishes.

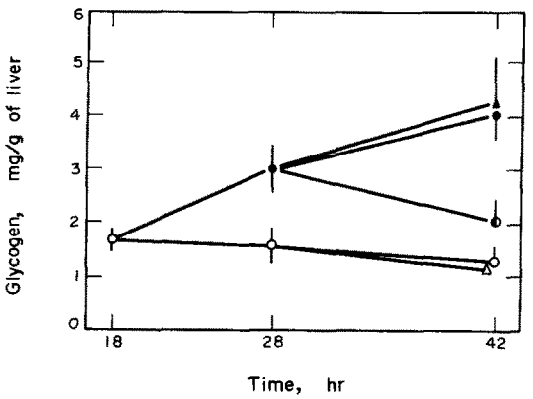

Fig. 4. Addition of betamethasone 17,21-dipropionate during progress of insulin-induced glycogen accumulation. Explants were exposed to insulin (4.0 μg/ml) (●) at 18 hr of culture. At 28 hr of culture, betamethasone 17,21-dipropionate (10^{-7} M) was added (▲, △) or insulin was removed (◑). Each point represents the mean ± S.D. of explants from five separate dishes.

one. However, this synthetic steroid may have a non-specific toxic effect on the explants. The effect of betamethasone 17,21-dipropionate on the morphology of explants was examined microscopically using H-E and PAS stains. Fresh fetal liver showed uniform distributions of hepatocytes and hematopoietic cell islands over the section, but the lobular structure of the hepatic cell cords was not obvious yet. PAS-positive materials in the hepatocytes were distributed homogeneously. There was a morphological difference between hepatocytes in the marginal and central areas during the culture period. By 18 hr hepatocytes in the marginal areas did not differ from those of fresh liver except for a slightly smaller amount of PAS-positive materials, but hepatocytes in the central area had degenerated. The cells in the central area were dispersed and had loose cellular connections, and the disappearance of hematopoietic cells was marked. These changes after 42 hr of culture were essentially the same as those after 18 hr, although the degenerated area was more widespread and the PAS-positive materials had decreased further. These morphological changes of explants during the culture period were not affected by the addition of betamethasone 17,21-dipropionate or cortisol, except that cortisol prevented the decrease in PAS-positive materials. These results and the fact that betamethasone 17,21-dipropionate did not interrupt the glycogen accumulation which already had been induced by insulin (Table 6) indicate that this steroid did not have a toxic effect on the explants.

DISCUSSION

Glucocorticoids play an important role in glycogen accumulation in fetal rat liver during late pregnancy [8]. In organ culture, glycogen accumulation in fetal rat liver does not occur in the absence of glucocorticoids or insulin [3, 4]. The optimum concentration of cortisol for glycogen accumulation was 10^{-7} M in our experimental system. Because the plasma level of corticosterone was about 30 μg/dl (8.7×10^{-7} M) in fetal rats on day 19 of gestation, the concentration in organ culture was well within the physiological range. Betamethasone 17,21-dipropionate suppressed cortisol-induced glycogen accumulation completely at 10^{-7} M and by about 40 per cent at 10^{-9} M; the accumulation was slight at 3×10^{-9} M. These results suggest that this steroid has a higher affinity than cortisol for the fetal liver.

The explants that were preincubated with betamethasone 17,21-dipropionate and were then transferred into medium containing cortisol, accumulated glycogen, which shows that the action of the synthetic steroid is reversible. In this case, however, the action caused by cortisol was about 54 per cent of the control preincubated without betamethasone 17,21-dipropionate. The liver is a major target organ of glucocorticoids and the only organ which concentrates these hormones *in vivo* above their blood level [9]. Because betamethasone 17,21-dipropionate is a synthetic glucocorticoid and its affinity for the liver may be high, it may be concentrated in the explants during preincubation and retained at a level high enough to suppress partially the action of cortisol, even after transfer of the explants to a medium without it.

Addition of betamethasone 17,21-dipropionate, during the process of cortisol-induced glycogen accumulation, interrupted and decreased the accumulation; this effect was the same as the effect of removal of cortisol. Cortisol does not affect the degradative pathway in cultured fetal rat hepatocytes [7]. Therefore, betamethasone 17,21-dipropionate should not stimulate the degradation of glycogen.

Betamethasone 17,21-dipropionate partially suppressed insulin-induced glycogen accumulation and preincubation with it did not affect the glycogenic action of insulin. The glycogenic process stimulated by insulin may not be as sensitive to the synthetic steroid as the process stimulated by cortisol. Betamethasone 17,21-dipropionate was effective when added simultaneously with insulin. It has been suggested that the glycogenic action of insulin is mediated by a stimulated conversion of glycogen sythase (EC 2.4.1.11) *b* to *a* in fetal rat liver [10, 11]. The results in experiments *in vivo* suggest that betamethasone 17,21-dipropionate may inhibit the conversion of glycogen synthase *b* to *a* [2]. This synthetic steroid may inhibit the first step of the conversion system of glycogen synthase.

The exact mechanism by which glucocorticoids stimulate glycogen synthesis in fetal rat liver is not known. It is accepted generally that the direct effects are mediated by the binding of the steroids to cytoplasmic steroid receptors. Betamethasone 17,21-dipropionate may compete with cortisol for binding to cytoplasmic receptors.

Acknowledgements—We thank Dr. T. Yoshizaki for his counsel and criticism throughout this study and Mr. Y. Muraoka for his morphological examination of explants.

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