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THE ROLE OF GLUCOCORTICOIDS IN THE REGULATION OF GLUCOSE-6-PHOSPHATASE ACTIVITY IN FETAL RAT LIVER

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Abstract—In organ culture of fetal rat liver explants, glucagon (5×10^{-5} M) or dibutyryl cyclic AMP (Bt_2cAMP) (5×10^{-5} M) increased glucose-6-phosphatase activity 2.4- to 2.8-fold. The Bt_2cAMP-induced increase in the enzyme activity was suppressed by cortisol at concentrations above 10^{-7} M. Betamethasone produced more potent suppression than cortisol did. Betamethasone 17,21-dipropionate enhanced the Bt_2cAMP action at 10^{-8} M but suppressed it weakly at concentrations above 10^{-7} M. With glucocorticoid alone, cortisol and betamethasone 17,21-dipropionate slightly increased the enzyme activity. Betamethasone 17,21-dipropionate blocked the suppression by cortisol of the Bt_2cAMP-induced increase in the enzyme activity. In experiments *in utero*, when glucocorticoids (100 μg/fetus) were injected s.c. into fetuses on day 19 of gestation, cortisol and corticosterone did not affect the age-dependent increase in glucose-6-phosphatase activity. Betamethasone 17,21-dipropionate stimulated this increase but betamethasone abolished it. The explanation of these results is that betamethasone 17,21-dipropionate and betamethasone antagonized endogenous corticosterone, resulting in a reduction by the former of the suppressive effect of corticosterone on the endogenous cAMP-mediated increase in the enzyme activity and a more intense suppression by the latter of the action of endogenous cAMP. Glucocorticoids may play an important role in the regulation of glucose-6-phosphatase activity by modulating the action of cAMP in fetal rat liver during late pregnancy.

The effects of various hormones on hepatic glucose-6-phosphatase (EC 3.1.3.9) activity have been well investigated in adult animals. Glucocorticoids and insulin participate mainly in the regulation of this activity, but the role of glucagon is not clear [1]. In fetal rat liver, glucose-6-phosphatase activity increases in the last few days of gestation and it has been suggested that glucagon and cAMP initiate this increase since their administration to fetuses *in utero* induces a premature increase in glucose-6-phosphatase activity [2, 3]; however, the enzyme does not respond to glucocorticoids [4, 5]. During the investigation of the inhibitory effect of betamethasone 17, 21-dipropionate on glycogen storage in fetal rat liver [6, 7], we found that it increased glucose-6-phosphatase activity, suggesting that glucocorticoids may participate in the regulation of the enzyme activity.

Glucocorticoids exhibit a permissive or synergistic action toward various effects of several hormones which appear to act cAMP [8–10]. We investigated the effect of glucocorticoids on the glucagon-or the dibutyryl cyclic AMP (Bt_2cAMP)-induced increase in glucose-6-phosphatase activity in fetal rat liver. The results show that glucocorticoids inhibit the increasing action of glucagon or Bt_2cAMP on the enzyme activity and suggest that corticosterone plays an important role in the regulation of glucose-6-phosphatase in fetal rat liver during late pregnancy.

MATERIALS AND METHODS

Animals. Pregnant rats of the JCL Sprague–Dawley strain (CLEA Japan, Inc.) were laparotomized under ether anesthesia and fetuses were injected s.c. with a suspension of steroid in 20 μl of 0.5% acacia gum solution on day 19 of gestation. At the appropriate time, the mothers were killed; the fetuses were removed immediately and the livers were excised and homogenized.

Organ culture. Livers were removed under sterile conditions from fetal rats on day 19 of gestation. Organ culture was carried out by a method described previously [7]. Briefly, about 1-mm cubes of explants were placed on stainless steel grids in Eagle's minimal essential medium (MEM) with Earle's balanced salt solution (BSS) and incubated in a humidified incubator at 37° with a circulating gas phase of 95% air–5% CO_2.

Assay of glucose-6-phosphatase. The assay mixture, in a final volume of 0.5 ml, contained 60 mM fumarate buffer (pH 6.2), 50 mM glucose-6-P, and 50 μl of homogenate. Incubation was carried out for 10 min at 30°. Released inorganic phosphate was determined by the method of Lindberg and Ernster [11]. A 10% homogenate was prepared in ice-cold 0.25 M sucrose and incubated with 0.2% deoxycholate for 10 min in an ice bath to activate the enzyme [12]. Protein concentration was determined by the method of Lowry *et al.* [13].

Chemicals. Glucagon (bovine pancreas, minimal insulin content) was purchased from CalBiochem (San Diego, CA, U.S.A.), dibutyryl cAMP monosodium salt and glucose-6-P disodium salt from Boehringer-Mannheim GmbH (Mannheim, West Germany), corticosterone, betamethasone and dexamethasone from the Sigma Chemical Co. (St. Louis, MO, U.S.A.), betamethasone 17,21-dipropionate from the Shering Corp. (Bloomfield, NJ, U.S.A.), cortisol and deoxycholate from Nakarai Chemicals Ltd. (Kyoto, Japan), and Eagle's MEM and Earle's BSS from the Nissui Seiyaku Co., Ltd. (Tokyo, Japan).

Table 1. Effects of cortisol on glucagon-induced increase in glucose-6-phosphatase activity in fetal rat liver in organ culture*

Addition		
Glucagon (M)	Cortisol (M)	Glucose-6-phosphatase (nmoles/min/mg protein)
0	0	16.2 ± 1.6
5×10^{-7}	0	24.5 ± 4.3†
5×10^{-6}	0	29.7 ± 3.9†
5×10^{-5}	0	38.1 ± 2.9†
0	10^{-6}	19.4 ± 1.5‡
5×10^{-5}	10^{-6}	28.2 ± 1.3†§

* Explants were incubated for 18 hr in control medium and then exposed to glucagon (bovine) and cortisol for 24 hr. Values are means ± S.D. of explants from five dishes.

† Significance of difference from control: $P < 0.01$.

‡ Significance of difference from control: $P < 0.05$.

§ Significance of difference from glucagon-treated explants: $P < 0.01$.

RESULTS

Effects of glucocorticoids on glucagon- or Bt_2cAMP-induced increase in glucose-6-phosphatase activity in explants. Table 1 shows the effects of glucagon and cortisol on glucose-6-phosphatase activity in fetal rat liver explants. Glucagon increased the enzyme activity at concentrations above 5×10^{-7} M, and the activity reached 2.4-fold the control value at 5×10^{-5} M. The glucagon concentrations required were the same as those used in the induction of tyrosine aminotransferase (EC 2.6.1.5) [4] and the arginine synthase system [14] in fetal rat liver explants. Cortisol (10^{-6} M) suppressed the glucagon-induced increase in glucose-6-phosphatase activity and the steroid alone weakly increased the enzyme activity.

Bt_2cAMP also increased glucose-6-phosphatase activity, and the activity almost reached the maximal level at 5×10^{-5} M as shown in Table 2. The effective Bt_2cAMP concentration was the same as that required for the induction of glucose-6-phosphatase in human fetal liver explants [15] and of tyrosine aminotransferase [4] and the arginine synthase system [14].

Cortisol suppressed the Bt_2cAMP (5×10^{-5} M)-induced increase in glucose-6-phosphatase activity at concentrations above 10^{-7} M; the suppression by

Table 2. Effects of dibutyryl cyclic AMP on glucose-6-phosphatase activity in fetal rat liver in organ culture*

Bt_2cAMP (M)	Glucose-6-phosphatase (nmoles/min/mg protein)
0	18.7 ± 1.6
10^{-6}	20.9 ± 1.6
5×10^{-6}	26.4 ± 2.6†
10^{-5}	28.6 ± 3.7†
5×10^{-5}	49.2 ± 7.5†
10^{-4}	55.5 ± 5.7†

* Explants were incubated for 18 hr in control medium and then exposed to dibutyryl cyclic AMP for 24 hr. Values are means ± S.D. of explants form five dishes.

† $P < 0.01$.

Table 3. Effects of cortisol on dibutyryl cyclic AMP-induced increase in glucose-6-phosphatase activity in fetal rat liver in organ culture*

Addition		
Bt_2cAMP (M)	Cortisol (M)	Glucososе-6-phosphatase (nmoles/min/mg protein)
0	0	12.6 ± 1.1
5×10^{-5}	0	31.6 ± 2.3†
0	10^{-8}	15.6 ± 1.2†
5×10^{-5}	10^{-8}	37.1 ± 3.4†‡
0	10^{-7}	17.2 ± 0.6†
5×10^{-5}	10^{-7}	25.4 ± 3.6†‡
0	10^{-6}	13.2 ± 1.0
5×10^{-5}	10^{-6}	17.4 ± 1.7†§

* Explants were incubated for 18 hr in control medium and then exposed to dibutyryl cyclic AMP and cortisol for 24 hr. Values are means ± S.D. of explants from five dishes.

† Significance of difference from control: $P < 0.01$.

‡ Significance of difference from dibutyryl cyclic AMP-treated explants: $P < 0.05$.

§ Significance of difference from dibutyryl cyclic AMP-treated explants: $P < 0.01$.

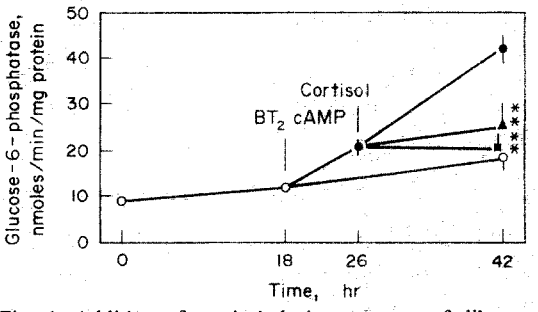

Fig. 1. Addition of cortisol during progress of dibutyryl cyclic AMP-induced increase in glucose-6-phosphatase activity in fetal rat liver in organ culture. Explants were exposed to 5×10^{-5} M Bt_2cAMP (●) at 18 hr of culture. At 26 hr of culture, cortisol (10^{-6} M) was added (▲) or Bt_2cAMP was removed (■). Each point represents the means ± S.D. of explants from five dishes. Significance of difference from corresponding Bt_2cAMP-treated explants: $^{**}P < 0.01$.

Table 4. Effects of betamethasone on dibutyryl cyclic AMP-induced increase in glucose-6-phosphatase activity in fetal rat liver in organ culture*

Addition		
Bt_2cAMP (M)	Betamethasone (M)	Glucose-6-phosphatase (nmoles/min/mg protein)
0	0	18.0 ± 3.2
5×10^{-5}	0	50.3 ± 8.8†
0	10^{-8}	20.7 ± 3.3
5×10^{-5}	10^{-8}	35.8 ± 3.3†‡
0	10^{-7}	19.7 ± 1.8
5×10^{-5}	10^{-7}	24.3 ± 2.4†‡
0	10^{-6}	22.1 ± 3.1
5×10^{-5}	10^{-6}	28.8 ± 3.3†‡

* Explants were incubated for 18 hr in control medium and then exposed to dibutyryl cyclic AMP and betamethasone for 24 hr. Values are means ± S.D. of explants from five dishes.

† Significance of difference from control: $P < 0.01$.

‡ Significance of difference from dibutyryl cyclic AMP-treated explants: $P < 0.01$.

Table 5. Effects of betamethasone 17,21-dipropionate on dibutyryl cyclic AMP-induced increase in glucose-6-phosphatase activity in fetal rat liver in organ culture*

Addition		
Bt_2cAMP (M)	Betamethasone 17,21-dipropionate (M)	Glucose-6-phosphatase (nmoles/min/mg protein)
0	0	21.1 ± 2.4
5×10^{-5}	0	59.8 ± 11.4†
0	10^{-8}	23.5 ± 5.0
5×10^{-5}	10^{-8}	73.6 ± 4.4†‡
0	10^{-7}	28.2 ± 3.2§
5×10^{-5}	10^{-7}	55.1 ± 4.4†
0	10^{-6}	33.1 ± 2.9†
5×10^{-5}	10^{-6}	58.9 ± 8.7†

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

† Significance of difference from control: $P < 0.01$.

‡ Significance of difference from dibutyryl cyclic AMP-treated explants: $P < 0.01$.

§ Significance of difference from control: $P < 0.05$.

10^{-6} M cortisol was 78 per cent (Table 3). At 10^{-8} M, cortisol did not suppress the action of Bt_2cAMP, and the increase by simultaneous addition of both agents was almost equal to the sum of those by the individual agents. A small increase in the enzyme activity by cortisol alone was not dose responsive. Corticosterone, equally, showed these two effects, like cortisol (data not shown). As shown in Fig. 1, cortisol interrupted the progress of the Bt_2cAMP-induced increase in glucose-6-phosphatase activity; this effect was similar to that of withdrawal of Bt_2cAMP.

The suppressive effect of betamethasone on the action of Bt_2cAMP was more potent than that of cortisol, with a 47 per cent decrease in the presence of 10^{-8} M betamethasone (Table 4). Betamethasone did not increase glucose-6-phosphatase activity.

Because betamethasone 17,21-dipropionate does not have glycogenic activity and inhibits the glycogenic action of cortisol in fetal rat liver explants [7], its effect on glucose-6-phosphatase activity was examined. Betamethasone 17,21-dipropionate at 10^{-8} M potentiated the effect of Bt_2cAMP on glucose-6-phosphatase activity (Table 5). At concentrations above 10^{-7} M, it did not apparently, suppress the action of Bt_2cAMP, but, because it increased the enzyme activity when given alone, its suppression of Bt_2cAMP action was obvious when the effect of Bt_2cAMP in its presence was calculated by subtracting a corresponding increase with betamethasone 17,21-dipropionate alone from an increase with it and Bt_2cAMP. The effect of simultaneous addition of cortisol and betamethasone 17,21-dipropionate on the Bt_2cAMP-induced increase in glucose-6-phosphatase activity was equal to that of betamethasone 17,21-dipropionate (Table 6), indicating that both steroids act on the same site and that the affinity of betamethasone 17,21-dipropionate for fetal rat liver is greater than that of cortisol.

Figure 2 shows the relative effects of three glucocor-

Table 6. Effects of simultaneous addition of cortisol and betamethasone 17,21-dipropionate on dibutyryl cyclic AMP-induced increase in glucose-6-phosphatase activity in fetal rat liver in organ culture*

Addition			
Bt_2cAMP (M)	Cortisol (M)	Betamethasone 17,21-dipropionate (M)	Glucose-6-phosphatase (nmoles/min/mg protein)
0	0	0	24.1 ± 2.2
5×10^{-5}	0	0	54.2 ± 4.8†
0	10^{-6}	0	28.3 ± 5.4
5×10^{-5}	10^{-6}	0	32.2 ± 2.1†‡
0	0	10^{-6}	32.4 ± 3.5†
5×10^{-5}	0	10^{-6}	48.7 ± 5.8†
0	10^{-6}	10^{-6}	31.4 ± 2.4†
5×10^{-5}	10^{-6}	10^{-6}	56.1 ± 8.0†

* Explants were incubated for 18 hr in control medium and then exposed to dibutyryl cyclic AMP and glucocorticoids for 24 hr. Values are means ± S.D. of five dishes.

† Significance of difference from control: $P < 0.01$.

‡ Significance of difference from dibutyryl cyclic AMP-treated explants: $P < 0.01$.

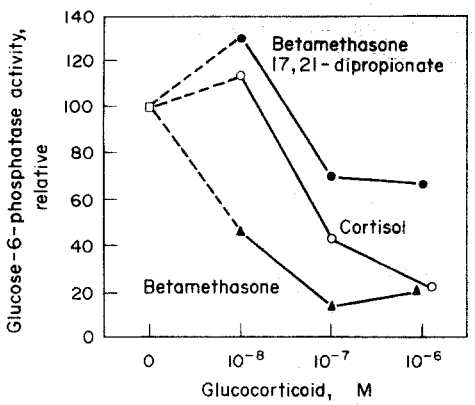

Fig. 2. Effects of glucocorticoids on dibutyryl cyclic AMP-induced increase in glucose-6-phosphatase activity in fetal rat liver in organ culture. Each value was calculated from the data in Tables 3, 4 and 5 by subtracting the corresponding increase with steroid alone from the increase with Bt_2cAMP plus steroid.

ticoids on the Bt_2cAMP-induced increase in glucose-6-phosphatase activity. At 10^{-7} M, the potency of suppressive action of steroids was as follows: betamethasone > cortisol > betamethasone 17,21-dipropionate.

Effects of glucocorticoids on glucose-6-phosphatase activity in vivo. Figure 3 shows the effects of betamethasone and its dipropionate on glucose-6-phosphatase activity in the liver when the fetuses were treated *in utero* with 100 μg of steroid on day 19 of gestation. Betamethasone completely abolished the age-dependent increase in glucose-6-phosphatase activity. Dexamethasone also showed the same effect as betamethasone, but cortisol and corticosterone did not affect the enzyme activity (data not shown). Betamethasone 17,21-dipropionate stimulated the age-dependent increase in enzyme activity.

DISCUSSION

Glucocorticoids showed two effects on the regulation of glucose-6-phosphatase activity in fetal rat liver explants. One was the increase in enzyme activity induced by glucocorticoid alone. This effect was somewhat less evident with cortisol, obvious with betamethasone 17,21-dipropionate, but not observed with betamethasone. The increasing action on glucose-6-phosphatase activity may be an intrinsic property of glucocorticoid, because cortisol and corticosterone, which are natural glucocorticoids, increased the enzyme activity. Adult rat liver glucose-6-phosphatase is activated by treatment with a detergent such as deoxycholate [12], and fetal rat liver enzyme was also weakly activated (unpublished observations). In adult rats, an increase in glucose-6-phosphatase activity caused by glucocorticoids is noted only when the assay is carried out in the absence of deoxycholate, no significant difference between glucocorticoid-treated and untreated groups being seen when the assay is carried out in its presence. This suggests that the effects of glucocorticoids are based on the activation of pre-existing enzyme [12]. In this study, because the assay was carried out in the presence of deoxycholate, the effects of cortisol and betamethasone 17,21-dipropionate may not be the result of activation of latent enzyme.

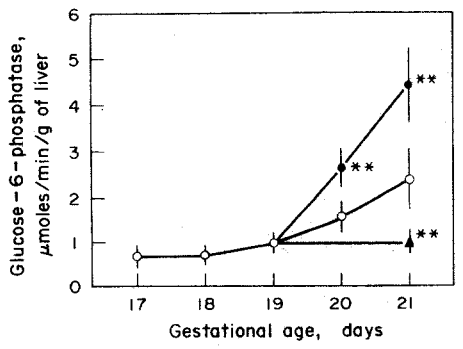

Fig. 3. Effects of betamethasone 17,21-dipropionate and betamethasone on glucose-6-phosphatase activity in fetal rat liver. The fetuses were injected s.c. *in utero* with 100 μg betamethasone 17,21-dipropionate (●) or betamethasone (▲) on day 19 of gestation. Each point represents the mean ± S.D. of eight fetuses from two litters. Significance of difference from control: $^{**}P < 0.01$.

The other noteworthy effect is suppression of the glucagon- or Bt_2cAMP-induced increase in glucose-6-phosphatase activity. Glucocorticoids generally exhibit a permissive or synergistic action towards glucagon- or cAMP-stimulated events, e.g. gluconeogenesis in the liver [8], amino acid uptake by isolated hepatocytes [16], and induction of tyrosine aminotransferase in isolated hepatocytes [17]. In fetal rat liver explants, while the simultaneous addition of cAMP and cortisol exhibits a marked synergistic effect on the induction of tyrosine aminotransferase [4], the combination of Bt_2cAMP with triamcinolone produces effects somewhat less than the additive one on the induction of the arginine synthase system [14]. Our results indicate that glucocorticoids suppress the action of Bt_2cAMP on glucose-6-phosphatase activity. Glucocorticoids either stimulate or inhibit individual cAMP responsive systems in fetal rat liver and the specificity of such reciprocal glucocorticoid action does not seem to be determined by the level of phosphodiesterase (EC 3.1.4.1), which is proposed as the site of the permissive effect of glucocorticoids in cultured rat hepatoma (HTC) cells [18]. Rousseau [19] suggests that the site of permissive action of glucocorticoids is at a level beyond the protein kinase (EC 2.7.1.37) system in the experiments with HTC cells.

Cortisol and corticosterone did not affect glucose-6-phosphatase activity *in vivo*. Because the plasma corticosterone level on day 19 of gestation was 30 μg/100 ml (8.7×10^{-7} M), which may be enough to stimulate fully glucocorticoid-responsive systems in the liver, exogenous cortisol or corticosterone may have been even more ineffective. Betamethasone 17,21-dipropionate had a greater affinity for the active site of the liver than cortisol, but its suppressive effect on the Bt_2cAMP-induced increase in glucose-6-phosphatase activity was weaker than that of cortisol. Thus, it may displace endogenous corticosterone from the active site and this may reduce the suppression of glucagon (or cAMP) action on glucose-6-phosphatase. Betamethasone may have a greater affinity for the active site of the liver and show a more potent suppressive effect on Bt_2cAMP action than corticosterone, which would

cause more severe suppression of the age-dependent increase in glucose-6-phosphatase activity.

A physiological concentration of cortisol suppressed the glucagon- or Bt_2cAMP-induced increase in glucose-6-phosphatase activity in fetal rat liver explants whereas betamethasone 17,21-dipropionate, which suppressed the action of Bt_2cAMP less effectively than cortisol *in vitro*, increased glucose-6-phosphatase activity *in vivo*. These results suggest that glucagon and glucocorticoids play important roles in the regulation of fetal rat liver glucose-6-phosphatase activity. During late pregnancy, the concentrations of glucagon in plasma begin to increase on day 18 of gestation (3×10^{-11} M), reach a maximum (12×10^{-11} M) on day 20, and then decrease to about 64 per cent of the maximal level on day 21 [20]. Plasma corticosterone concentrations begin to increase on day 17, reach the maximum on day 19, and then decrease thereafter [21]. The fact that glucose-6-phosphatase activity begins to increase on day 20 when the glucagon level reaches the maximum and the corticosterone level begins to decrease coincides with the assumption described above. The plasma levels of norepinephrine and epinephrine in fetal rats on the last day of gestation are the same as those in adult rats [22] and administration of epinephrine increases the glucose-6-phosphatase activity in fetal rat liver [3]. The balance of glucagon, catecholamines and corticosterone may be important in the age-dependent increase in glucose-6-phosphatase in fetal rat liver.

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