Role of dexamethasone and insulin on the development of the five urea-cycle enzymes in cultured rat foetal hepatocytes

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The activity changes of the urea-cycle enzymes were monitored in cultured foetal hepatocytes after dexamethasone and insulin treatments. Addition of dexamethasone induced the development of carbamoyl-phosphate synthetase, argininosuccinate synthetase, argininosuccinase and arginase activities as soon as day 16.5 of gestation. When insulin was added together with dexamethasone, it markedly inhibited the steroid-induced increase in carbamoyl-phosphate synthetase, argininosuccinate synthetase and argininosuccinase activities.

It is well documented that general protein degradation and urea synthesis are stimulated by glucagon and inhibited by insulin (Miller, 1961; Exton & Park, 1972). In particular, glucagon administration (McLean & Novello, 1965; Snodgrass *et al.*, 1978), diabetes (Jorda *et al.*, 1981) or starvation (Schimke, 1962) lead to an increase in urea-cycle enzyme activities in adult rat liver, although no effect of insulin has been found *in vivo* (McLean & Novello, 1965) or *in vitro* (Lin *et al.*, 1982).

Insulin and glucagon are secreted during the late-foetal period in the rat (Felix *et al.*, 1969; Girard *et al.*, 1971), and it was shown that circulating insulin concentrations are high in the foetus and fall soon after birth. The five urea-cycle enzyme activities, namely CPS, OTC, ASS, ASL and arginase (EC 3.5.3.1), are low during the foetal period and increase rapidly during the first day of life (Räihä & Suihkonen, 1968; Gautier *et al.*, 1977). These data suggested that the low enzyme activities during foetal life could result from the effect of the hormones secreted at this stage of development. Previous studies with whole foetal-rat livers indicated that CPS, OTC, ASS and arginase could not develop without glucocorticoids, and ASL was the only enzyme of the cycle not affected by steroid addition or deprivation before term (Husson & Vaillant, 1979b). Moreover, ASL activity could be induced pre-

turely by administration of glucagon (or dibutyryl cyclic AMP), and glucocorticoids had a synergistic effect on this action (Husson & Vaillant, 1982).

In primary cultured foetal hepatocytes, the ASS, ASL and arginase activities were maintained at their initial values for a few days (Husson *et al.*, 1983, 1984). Dexamethasone and glucagon independently increased ASL and arginase activities and acted synergistically when added in combination to the culture medium (Husson *et al.*, 1984).

The present study was undertaken to determine the role of dexamethasone and insulin in the regulation of the five urea-cycle enzyme activities during foetal life.

Materials and methods

Materials

Animals. Adult female rats (Wistar strain) were mated overnight and recognized as pregnant by a vaginal smear on the next morning. Parturition occurred at 21.5 days.

Chemicals. The materials used for cell isolation and culture were as reported previously (Husson *et al.*, 1983). The culture medium was a mixture of Eagle's Minimal Essential Medium and Medium 199 with Hanks salts, deficient in arginine, obtained from Eurobio (Paris, France). Insulin and dexamethasone were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Methods

Culture of foetal-rat hepatocytes. Hepatocytes from 15.5–19.5-day fetuses were cultured by the method of Leffert & Paul (1972) with some

Abbreviations used: CPS, carbamoyl-phosphate synthetase (EC 6.3.4.16); OTC, ornithine transcarbamoylase (EC 2.1.3.3); ASS, argininosuccinate synthetase (EC 6.3.4.5); ASL, argininosuccinase (EC 4.3.2.1).

modifications (Husson *et al.*, 1984). The cells were suspended in the culture growth medium containing 10% (v/v) charcoal-treated foetal-calf serum and cultured in 25 cm² tissue-culture flasks at 37°C under 5% CO₂ in air. After an attachment period of 4–5 h and every 24 h thereafter, the medium was replaced by fresh medium with 5% foetal-calf serum and hormones. Cells were harvested and homogenized after 3 days of culture.

Assays of urea-cycle enzyme activities. All enzyme assays were made on fresh extracts prepared as described previously (Husson *et al.*, 1983). Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. CPS and OTC activities were assayed by the method of Brown & Cohen (1959). ASS and ASL activities were determined by the procedures of Ratner (1955), and arginase was assayed as described by McLean & Gurney (1963).

One unit of urea-cycle enzyme activity is defined as the production of 1 μ mol of product/h at 38°C. Results are expressed as means $\pm$ S.E.M.. The numbers of observations being given in parentheses. Significance was determined by using a paired Student's *t* test.

Results and discussion

In previous studies, it was shown that foetal hepatocytes in culture constitute an ideal tool for studying the developmental regulatory mechanisms of ASS, ASL and arginase activities (Husson *et al.*, 1983, 1984). To specify this phenomenon

further, we measured the five urea-cycle enzyme activities in hepatocytes cultured at different stages of maturation. From the available data, it has been shown that the younger the foetal cells are, the more actively they synthesize enzymes (Guguen-Guillouzo *et al.*, 1980).

Effects of dexamethasone

Cells from 15.5–19.5-day fetuses were cultured for 3 days with dexamethasone, and the requirements of the enzyme activities for the glucocorticoid inducer are compared in Table 1. At every stage studied, CPS activity rose after dexamethasone addition. These results are in agreement with previous studies *in vivo*, where cortisol administration on 16.5-day fetuses produced a 2-fold increase in activity 24 h later (Gautier *et al.*, 1977).

OTC is the only enzyme of the cycle that was never changed by steroid addition to the culture medium. Previous studies had shown that this activity was greatly depressed in corticosteroid-deprived fetuses (Gautier *et al.*, 1977), but could not be induced by cortisol administration to intact fetuses. Hypophysectomy of fetuses rendered OTC inducible by cortisol, whereas with foetal hepatocytes in culture reproducible induction by the steroid could not be obtained. This result is in agreement with the observation by Edkins & Rih (1976) on foetal-liver explants and suggests that the development of OTC activity is not directly under the glucocorticoid control.

The effect of dexamethasone in increasing the

Table 1. *Effects of dexamethasone on urea-cycle enzyme activities in cultured foetal hepatocytes*
Hepatocytes from 15.5–19.5-day fetuses were cultured during 3 days without or with dexamethasone (1 μ M; dexa). Values are means $\pm$ S.E.M. for the numbers of independent experiments from separate animals indicated in parentheses. Abbreviation: nd, not detected.

Foetus age (days)	Culture conditions	Activity (μ mol/h per mg of protein)				
		CPS	OTC	ASS	ASL	Arginase
15.5	Control	nd (2)	47.7 (2) (42.3, 53.1)	0.37 (2) (0.32, 0.42)	nd (2)	8.4 (2) (6.0, 10.8)
	Dexa	0.46 (2) (0.41, 0.51)	36.1 (2) (31.3, 40.9)	0.62 (2) (0.59, 0.65)	0.32 (2) (0.28, 0.36)	100.5 (2) (78.8, 122.3)
16.5	Control	0.19 $\pm$ 0.05 (3)	80.0 $\pm$ 13.4 (4)	0.44 $\pm$ 0.08 (4)	0.13 $\pm$ 0.02 (3)	46.1 $\pm$ 8.1 (9)
	Dexa	0.48 $\pm$ 0.06 (3)	75.1 $\pm$ 21.9 (4)	0.81 $\pm$ 0.07 (4)	0.36 $\pm$ 0.05 (3)	261.7 $\pm$ 19.1 (9)
17.5	Control	0.25 $\pm$ 0.05 (4)	69.6 $\pm$ 10.5 (4)	0.47 $\pm$ 0.02 (7)	0.14 $\pm$ 0.01 (7)	63.0 $\pm$ 8.2 (9)
	Dexa	0.69 $\pm$ 0.08 (4)	66.3 $\pm$ 10.6 (4)	0.97 $\pm$ 0.08 (7)	0.30 $\pm$ 0.02 (7)	274.3 $\pm$ 31.3 (9)
18.5	Control	0.37 $\pm$ 0.07 (5)	70.7 $\pm$ 9.7 (4)	0.68 $\pm$ 0.08 (10)	0.19 $\pm$ 0.01 (10)	72.7 $\pm$ 8.0 (10)
	Dexa	0.97 $\pm$ 0.05 (5)	65.1 $\pm$ 9.5 (7)	1.16 $\pm$ 0.16 (10)	0.54 $\pm$ 0.04 (10)	250.8 $\pm$ 30.1 (10)
19.5	Control	0.43 (2) (0.39, 0.47)	92.3 $\pm$ 10.7 (6)	0.99 $\pm$ 0.11 (5)	0.21 $\pm$ 0.01 (5)	110.0 $\pm$ 16.6 (6)
	Dexa	1.00 (2) (0.87, 1.13)	95.2 $\pm$ 11.8 (6)	1.53 $\pm$ 0.20 (4)	0.34 $\pm$ 0.03 (5)	218.3 $\pm$ 25.8 (6)

specific activities of ASS and ASL in cultured foetal hepatocytes as early as day 15.5 and day 16.5 respectively was found to be different from this after administration of cortisol *in utero* (Husson & Vaillant, 1982). Although ASS activity was greatly depressed in hypophysectomized foetuses, a lack of effect of cortisol on both enzyme activities from intact foetuses had been previously observed (Schwartz, 1972; Husson & Vaillant, 1979b). These results suggest that a suppressive phenomenon might counteract *in utero* the stimulation of these enzyme activities induced by the corticosteroids (Räihä & Edkins, 1977).

In 15.5-day hepatocytes no basal CPS and ASL activities could be detected, but, when dexamethasone was added, the enzyme activities were at the same values as those of 16.5-day treated cells.

The inducing effect of dexamethasone on the specific activity of arginase in cultured foetal hepatocytes was found to be close to this after administration of cortisol *in utero* (Husson & Vaillant, 1979a). The intensity of the response of the enzyme activity to the inducer was directly related to the stage of the cells used for the treatment. In 15.5-day cells, the activity was 12-fold higher in treated cells than in the paired controls, although the glucocorticoid-receptor activity has been shown to be very low at this stage (Cake *et al.*, 1981). The inducibility decreased noticeably in older stages and, when 19.5-day hepatocytes were used, the activity was only 1.9 times higher in treated cells than in paired controls. In general, arginase was the most responsive when compared with the four other enzymes (Table 1). This is the first report on the activity changes of the five urea-cycle enzymes in cultured foetal hepatocytes, and it can be seen that glucocorticosteroids act very differently on their development. Particularly, dexamethasone produces an increase in CPS, ASS, ASL and arginase activities at a precocious stage of liver organo-

genesis, even before the formation of functional adrenal glands.

The above results on urea-cycle enzymes during ontogenic development are quite different from those obtained in cultured adult hepatocytes, known to be less sensitive to dexamethasone alone (Gebhardt & Mecke, 1979; Lin *et al.*, 1982). The reason for this loss of sensitivity of cultured mature cells remains unclear, and the difference between foetal and mature hepatocytes in the mechanism of response to steroid stimulation requires investigation.

Action of insulin

During development, insulin stimulates or represses the activity of a number of enzymes, but little is known about its action on urea-cycle enzymes. Hepatocytes from 18.5-day foetuses were cultured for 3 days with insulin and with or without dexamethasone (Table 2).

Insulin when added alone had no significant effect on the enzyme activities, but, when incubated with dexamethasone, it produced a marked inhibitory effect on the steroid-induced increase of CPS, ASS and ASL activities. Particularly, the increase in ASS activity was completely abolished. These results are in agreement with the observations by Räihä & Edkins (1977) on ASS and ASL activities from foetal liver explants. OTC activity was not significantly changed by insulin addition. Arginase seems to be quite different from the others, since it was rather slightly increased after the hormone addition. However, the response of the enzyme activity was not synergistic when the two effectors were associated. Such an effect was also reported by McLean & Novello (1965) in adult-rat liver *in vivo*, and Grazi *et al.* (1972) have shown that insulin administration produced a large increase in arginase activity in chicken liver.

Several studies reported a similar antagonistic interaction of insulin and glucocorticoids, in

Table 2. Effects of insulin on dexamethasone-induced enzyme activities in cultured hepatocytes derived from 18.5-day foetuses. Cells were maintained for 3 days in control medium or in the presence of the specified compounds. Values are means $\pm$ S.E.M. for the numbers of separate cultures in parentheses: ^a $P < 0.01$ versus control; ^b $P < 0.05$ versus control; ^c $P < 0.01$ versus dexamethasone alone.

Culture conditions ...	Enzyme activities ($\mu\text{mol/h per mg of protein}$)			
	Control	Dexamethasone (1 μM)	Insulin (0.1 μM)	Dexamethasone (1 μM) + insulin (0.1 μM)
CPS	0.37 $\pm$ 0.07 (5)	0.97 $\pm$ 0.04 ^a (5)	0.39 $\pm$ 0.09 (4)	0.62 $\pm$ 0.04 ^c (6)
OTC	70.7 $\pm$ 9.7 (7)	65.1 $\pm$ 9.5 (7)	60.5 $\pm$ 10.3 (7)	67.6 $\pm$ 9.2 (8)
ASS	0.50 $\pm$ 0.06 (7)	0.82 $\pm$ 0.05 ^a (7)	0.45 $\pm$ 0.06 (6)	0.49 $\pm$ 0.03 ^c (8)
ASL	0.18 $\pm$ 0.01 (5)	0.44 $\pm$ 0.04 ^a (5)	0.19 $\pm$ 0.02 (5)	0.26 $\pm$ 0.02 ^c (7)
Arginase	63.0 $\pm$ 6.7 (7)	230.0 $\pm$ 21.4 ^a (7)	91.7 $\pm$ 13.6 ^b (7)	222.4 $\pm$ 30.8 (7)

Reuber H35 cells (Wicks *et al.*, 1974) and in foetal or adult hepatocytes (Ho *et al.*, 1981; Nakamura *et al.*, 1980). By contrast, potentiation of insulin action by glucocorticoids has been also reported (Plas & Nunez, 1976; Amatruda *et al.*, 1983). The physiological meaning of these interactions remains unclear, and the mechanism by which insulin antagonizes or potentiates glucocorticoid action is not yet known. RNA synthesis has been shown to be implicated in the induction of urea-cycle enzyme activities by glucocorticoids (Schwartz, 1972; Husson *et al.*, 1984), and it is possible that insulin produces a transcriptional or post-transcriptional block (Andreone *et al.*, 1982). The present data confirm and extend previous indications suggesting that expression of some enzymes is repressed before birth (Räihä & Edkins, 1977; Ho *et al.*, 1981). Furthermore, they suggest that the high plasma insulin concentration may repress the development of CPS, ASS and ASL activities during foetal life. The fall in this concentration, and the plasma corticosterone release at birth (Corbier & Roffi, 1974), could explain the rapid postnatal increase in these enzyme activities.

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