Relationship between lipogenesis and glycogen synthesis in maternal and foetal tissues during late gestation in the rat

Effect of dexamethasone

Manuel BENITO,*† Margarita LORENZO* and José María MEDINA‡

*Departamento de Bioquímica y Biología Molecular, Centro de Biología Molecular, C.S.I.C.,
Universidad Autónoma de Madrid, Madrid, and ‡Departamento de Bioquímica, Facultad de Farmacia,
Universidad de Salamanca, Salamanca, Spain

(Received 22 February 1982/Accepted 1 April 1982)

Treatment with dexamethasone enhanced $^3\text{H}_2\text{O}$ incorporation into liver and blood lipid, and also increased plasma glucose, insulin, non-esterified fatty acid and triacylglycerol concentrations during late gestation in the mother rat. An inverse relationship between glycogen and lipid synthesis in foetal liver and lung was observed in control rats. This relationship was also observed in foetal liver, but not in foetal lung, after treatment with dexamethasone.

During late gestation, profound changes occur in the concentration of lipid in maternal blood, which may be due to the changes in the rate of lipogenesis *de novo* observed in those circumstances (Scow *et al.*, 1964; Knoop *et al.*, 1973; Lorenzo *et al.*, 1981). Since the incorporation of maternal lipid into foetal rat tissues is likely to be secondary to lipogenesis *de novo* from maternal carbohydrates, the increase in plasma lipid during the second phase of gestation results in more glucose being available for foetal growth. However, the accumulation of glycogen in foetal liver during the last days of gestation (Devos & Hers, 1974) may contribute to the decrease in glucose availability for foetal and maternal lipogenesis. Actually, the decrease in lipogenesis in maternal and foetal liver observed during the last 3 days of gestation (Lorenzo *et al.*, 1981) may be concurrent with the increase in glycogen synthesis in foetal liver (Devos & Hers, 1974).

Treatment with glucocorticoids causes hyperlipidaemia and hyperinsulinaemia in non-pregnant rats. The hyperlipidaemia may be due to the increased lipogenic capacity in the liver as a consequence of the enhancement of the activity of the key enzymes for fatty acid synthesis (Krausz *et al.*, 1981). On the other hand, treatment with glucocorticoids increases the activity of glycogen synthase, which results in an increase in glycogen content of foetal-rat liver (Eisen *et al.*, 1973; Vanstapel *et al.*, 1980).

The aim of the present work was to study the effect of dexamethasone on the regulation of liver

† To whom reprint requests should be addressed.

lipogenesis *in vivo* during late gestation. Since dexamethasone crosses the placental barrier freely, a possible relationship between glycogen synthesis and lipogenesis in foetal tissues during late gestation has also been investigated.

Experimental

Albino Wistar pregnant rats (300–350 g) fed on a stock laboratory diet were injected with 0.2 ml of 0.9% NaCl or dexamethasone (0.8 mg/animal per day) between day 18 of gestation and the day before they were killed, at 20, 21 or 22 days. Conception was assumed by the presence of spermatozoa in the vagina, and gestational age was confirmed by the foetal weight. Rats were injected intraperitoneally with 5 mCi of $^3\text{H}_2\text{O}$ (The Radiochemical Centre, Amersham, Bucks., U.K.). After 50 min rats were anaesthetized with Nembutal (50 mg/kg body wt.), and 10 min later the abdomen was opened and maternal blood was collected from the aorta. Foetuses were delivered by rapid hysterectomy and killed by decapitation, exsanguinated by using heparinized capillary pipettes, and foetal plasma was separated by centrifugation. Then samples of maternal and foetal tissues were taken. Samples of tissue (1 g) or of heparinized blood (1 ml) were added to 3 ml of 30% (w/v) KOH, and the lipid was saponified and extracted by the method of Stansbie *et al.* (1976). In addition, frozen tissue powder (1 g) was extracted with 3 ml of 6% (w/v) HClO_4 and centrifuged at 2000 g for 15 min. Glycogen was precipitated in 2 ml of the HClO_4 extract of the

tissue at 4°C with 4 ml of ethanol. The glycogen pellet was washed, hydrolysed and freeze-dried as described by Postle & Bloxham (1980).

Plasma insulin was measured by radioimmunoassay by the method of Hales & Randle (1963), with human insulin as the standard. Non-esterified plasma fatty acids were assayed by the colorimetric method of Itaya & Ui (1965), and plasma triacylglycerols were extracted and saponified by the method of Eggstein & Kreutz (1966). Free glycerol was measured by enzymic assay (Garland & Randle, 1962). Neutralized HClO₄ extracts were used for the determination of glucose (Krebs *et al.*, 1963, 1964).

Results and discussion

Rates of lipogenesis in maternal tissues

Treatment with dexamethasone increased the rate of maternal liver lipogenesis and the incorporation of ³H₂O into blood lipid at 20, 21 and 22 days of gestation (Table 1). However, dexamethasone treatment did not affect the inhibition of the rate of hepatic lipogenesis observed during late gestation. At 22 days, this reached values as high as those found at 20 days in control rats (Table 1).

The enhancement of the rate of hepatic lipogenesis *in vivo* caused by dexamethasone in pregnant rats (Table 1) is in agreement with the reported effects of dexamethasone in virgin rats (Krausz *et al.*, 1981). These results may account for the significantly higher concentration of plasma triacylglycerol found after dexamethasone treatment as compared with untreated pregnant rats (Table 2). On the other hand, treatment with dexamethasone increased plasma

non-esterified fatty acid concentrations observed between 20 and 22 days of gestation (Table 2). These results may be explained by a permissive action or potentiating effect of dexamethasone on hormone-induced lipogenesis in adipose tissue as described by Kawai & Kuzuya (1981). The administration of dexamethasone increased plasma insulin concentration at 20–22 days of gestation (Table 2). As a result, a high concentration of plasma insulin was maintained during late gestation. These results seem to support the idea that dexamethasone works on the rate of lipogenesis *in vivo* in the liver through its capacity to induce insulin secretion by a direct effect on pancreatic cells (Krausz *et al.*, 1981; Svec & Rudis, 1981). However, the rate of lipogenesis in parametrial adipose tissue was not affected by this treatment (results not shown), which may be due to the marked resistance to insulin observed in rat adipose tissue during late gestation (Costrini & Kalkhoff, 1971; Flint *et al.*, 1979). The resistance to insulin of adipose tissue may also explain why dexamethasone increased release of non-esterified fatty acids as well as increasing plasma insulin concentrations (Table 2).

Treatment with dexamethasone enhanced the concentration of plasma glucose observed during the last 3 days of gestation despite the occurrence of hyperinsulinaemia in the same circumstances (Table 2). This result may be explained by the decrease in glucose utilization by peripheral tissues caused by the observed rise in plasma non-esterified fatty acids and/or by hepatic gluconeogenesis being stimulated through an activation of regulatory enzymes brought about by glucocorticoid treatment (Randle *et al.*, 1963; Krausz *et al.*, 1981).

Table 1. Effect of treatment with dexamethasone on the rates of lipogenesis and glycogen synthesis *in vivo* in maternal and foetal tissues during late gestation in the rat

For details see the Experimental section. The results are means $\pm$ S.E.M. ($n = 6-15$). Rates of lipogenesis and glycogen synthesis are expressed as μmol of ³H₂O incorporated into lipid or glycogen/h per g wet wt. of tissue or per ml of whole blood. Values that are significantly different by Student's *t* test from those for control rats on the corresponding day of gestation are shown: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	Treatment Period of gestation (days)	...	Control			Dexamethasone		
		...	20	21	22	20	21	22
Lipogenesis								
Maternal tissues								
Blood			3.1 ± 0.4	2.8 ± 0.5	1.8 ± 0.2	4.5 ± 0.6*	4.3 ± 0.6*	4.2 ± 0.8*
Liver			26.7 ± 1.7	18.9 ± 0.8	13.6 ± 1.0	45.0 ± 4.0***	30.4 ± 1.5***	23.8 ± 1.0***
Foetal tissues								
Placenta			4.9 ± 0.6	4.0 ± 0.2	4.1 ± 0.3	7.8 ± 0.7*	5.3 ± 0.5*	5.6 ± 0.4*
Liver			16.0 ± 0.7	12.0 ± 0.8	8.8 ± 0.4	9.7 ± 0.7***	7.3 ± 0.6***	5.6 ± 0.5***
Lung			6.4 ± 0.3	8.2 ± 0.9	8.9 ± 0.5	6.7 ± 0.6	5.7 ± 0.6***	5.4 ± 0.3***
Glycogen synthesis								
Foetal tissues								
Liver			14.0 ± 0.9	16.2 ± 1.0	5.2 ± 0.4	21.8 ± 2.3*	34.2 ± 4.0**	7.5 ± 0.7**
Lung			9.3 ± 0.7	4.6 ± 0.6	3.1 ± 0.4	4.9 ± 0.6***	2.7 ± 0.2**	3.6 ± 0.4

Table 2. *Effect of treatment with dexamethasone on the concentrations of metabolites and insulin in plasma during late gestation in the rat*

Blood from the maternal aorta, or whole foetal blood, was collected and the metabolites and insulin were determined as described in the Experimental section. The results are means $\pm$ S.E.M. ($n = 6-16$). Values that are significantly different by Student's *t* test from the corresponding day of gestation for control rats are shown: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Treatment Period of gestation (days) ...	Control			Dexamethasone		
	20	21	22	20	21	22
Maternal plasma						
Glucose ($\mu\text{mol/ml}$)	4.2 ± 0.3	4.4 ± 0.3	3.5 ± 0.4	$4.8 \pm 0.3^*$	$6.2 \pm 0.6^{**}$	$7.4 \pm 0.6^{***}$
Non-esterified fatty acids ($\mu\text{mol/ml}$)	1.6 ± 0.1	1.9 ± 0.3	0.9 ± 0.1	$2.9 \pm 0.2^{***}$	$2.9 \pm 0.2^{**}$	$2.6 \pm 0.2^{**}$
Triacylglycerols ($\mu\text{mol/ml}$)	5.1 ± 0.3	5.6 ± 0.5	1.7 ± 0.1	6.1 ± 0.5	$11.3 \pm 2.0^{**}$	$10.8 \pm 1.6^{***}$
Insulin ($\mu\text{units/ml}$)	110 ± 11	73 ± 11	27 ± 8	$211 \pm 28^{**}$	$223 \pm 13^{**}$	$219 \pm 7^{***}$
Foetal plasma						
Glucose ($\mu\text{mol/ml}$)	1.05 ± 0.11	1.37 ± 0.10	2.10 ± 0.22	1.58 ± 0.38	1.66 ± 0.15	2.40 ± 0.43
Insulin ($\mu\text{units/ml}$)	72 ± 9	128 ± 9	90 ± 7	62 ± 5	$78 \pm 4^{***}$	77 ± 11

Rates of lipogenesis and glycogen synthesis in foetal tissues

The rates of glycogen synthesis in foetal liver were 3-fold higher during days 20 and 21 of gestation as compared with those values in pregnant rats (results not shown), but decreased sharply on day 22 of gestation (Table 1). These results are in agreement with the storage of glycogen observed in foetal liver during the last 3 days of gestation (Devos & Hers, 1974). In addition, the rate of glycogen synthesis decreased sharply during the last day of gestation, when glycogenolysis is likely to be stimulated by the initiation of the increase in the plasma glucagon/insulin ratio observed in the rat at birth (Di Marco *et al.*, 1978; Martín *et al.*, 1981). Treatment with dexamethasone increased the rate of glycogen synthesis in foetal liver during the last 3 days of gestation, although the profile observed in the absence of treatment remained unchanged (Table 1). Conversely, the rate of lipogenesis in foetal liver decreased after dexamethasone treatment, as compared with the corresponding day of gestation of control pregnant rats (Table 1). These results seem to suggest the occurrence of an inverse relationship between lipogenesis and glycogen synthesis in foetal-rat liver during late gestation. Actually the increase in the rate of hepatic glycogen synthesis by dexamethasone may result in a decrease in glucose availability for lipogenesis in foetal liver.

The rate of lipogenesis in foetal lung increased during days 20–21 without any change during the last day of gestation (Table 1; Lorenzo *et al.*, 1981). Conversely, the rate of glycogen synthesis in foetal lung decreased during days 20–21, remaining unchanged during the last day of gestation (Table 1). Moreover, the decrease in the rate of glycogen synthesis was concurrent with the glycogen depletion that occurred in foetal-rat lung before birth

(Maniscalco *et al.*, 1978), a fact consistent with the suggestion that glycogen depletion provides glucose available for lipid synthesis in the lung (Maniscalco *et al.*, 1978). Therefore it may be suggested that there is an inverse relationship between glycogen and lipid synthesis in foetal-rat lung during late gestation. Treatment with dexamethasone decreased the rates of both lipogenesis and glycogen synthesis in foetal lung as compared with those values for foetuses from untreated pregnant rats (Table 1).

This inhibition of synthesis of both glycogen and lipid in the lung might be explained by a decrease in glucose utilization owing to the stimulation of lung lipoprotein lipase activity associated with the effect of corticoids on surfactant synthesis (Mostello *et al.*, 1981). In addition, the lack of effect of dexamethasone on glycogen synthesis may suggest that glucocorticoids could be ineffective in increasing glycogen synthase activity in foetal lung.

It is noteworthy that treatment with dexamethasone increased maternal, but not foetal, insulinaemia, a fact consistent with the idea that the mechanism for insulin secretion mediated by corticoids has not yet been developed in foetal pancreas (Krausz *et al.*, 1981; Svec & Rudis, 1981).

In conclusion, our results suggest the occurrence of an inverse relationship between glycogen and lipid synthesis in foetal liver and lung. This relationship was also observed in foetal liver when glycogen synthesis was enhanced by treatment with dexamethasone. On the other hand, treatment with dexamethasone enhanced liver lipogenesis and plasma lipid and glucose concentrations, increasing fuel supply to maternal and foetal tissues.

We are indebted to Professor F. Mayor for his encouragement and to Dr. T. Caldés for her assistance in the insulin assay. This work was supported by a grant

from the 'Foundation R. Areces', Spain. The 'Centro de Biología Molecular' was the recipient of a grant from 'F.I.S., Ministerio de Trabajo, Sanidad y Seguridad Social'.

References

- Costrini, N. V. & Kalkhoff, R. K. (1971) *J. Clin. Invest.* **50**, 992–998
- Devos, P. & Hers, H. G. (1974) *Biochem. J.* **140**, 331–340
- Di Marco, P. N., Ghisalverti, A. Y., Martin, C. E. & Oliver, I. T. (1978) *Eur. J. Biochem.* **87**, 243–247
- Eggstein, M. & Kreutz, F. H. (1966) *Klin. Wochenschr.* **44**, 262–266
- Eisen, H. J., Goldfine, I. D. & Glinsmann, W. H. (1973) *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3454–3457
- Flint, D. J., Sinnett-Smith, P. A., Clegg, R. A. & Vernon, R. G. (1979) *Biochem. J.* **182**, 421–427
- Garland, P. B. & Randle, P. J. (1962) *Nature (London)* **196**, 987–988
- Hales, C. N. & Randle, P. J. (1963) *Biochem. J.* **88**, 137–146
- Itaya, K. & Ui, M. (1965) *J. Lipid Res.* **6**, 16–20
- Kawai, A. & Kuzuya, N. (1981) *Horm. Metab. Res.* **13**, 224–228
- Knoop, R. H., Saudek, C. D., Arky, R. A. & O'Sullivan, J. B. (1973) *Endocrinology* **92**, 984–988
- Krausz, Y., Baron, H. & Shafir, E. (1981) *Biochim. Biophys. Acta* **663**, 69–82
- Krebs, H. A., Bennett, D. A. H., De Gasquet, P., Gascoyne, T. & Yoshida, T. (1963) *Biochem. J.* **86**, 22–27
- Krebs, H. A., Dierks, C. & Gascoyne, T. (1964) *Biochem. J.* **93**, 112–121
- Lorenzo, M., Caldés, T., Benito, M. & Medina, J. M. (1981) *Biochem. J.* **198**, 425–428
- Maniscalco, W. M., Wilson, C. M., Gross, I., Gobran, L., Rooney, S. A. & Warshaw, J. B. (1978) *Biochim. Biophys. Acta* **530**, 333–346
- Martin, A., Caldés, T., Benito, M. & Medina, J. M. (1981) *Biochim. Biophys. Acta* **672**, 262–267
- Mostello, D. J., Hamosh, M. & Hamosh, P. (1981) *Biol. Neonate* **40**, 121–128
- Postle, A. D. & Bloxham, D. P. (1980) *Biochem. J.* **192**, 65–73
- Randle, P. J., Garland, P. B., Hales, C. N. & Newsholme, E. A. (1963) *Lancet* **i**, 785–789
- Scow, R. O., Chernick, S. S. & Brinley, M. S. (1964) *Am. J. Physiol.* **206**, 796–804
- Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) *Biochem. J.* **160**, 413–416
- Svec, F. & Rudis, M. (1981) *Biochim. Biophys. Acta* **674**, 30–36
- Vanstapel, F., Doperé, F. & Stalmans, W. (1980) *Biochem. J.* **192**, 607–612