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The role of prolactin and progesterone in the regulation of lipogenesis in maternal and foetal rat liver *in vivo* and in isolated hepatocytes during the last day of gestation

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The administration of progesterone on day 21 of gestation increases the rates of lipogenesis in the liver *in vivo* and in hepatocytes isolated from rats on day 22 of pregnancy. Bromocriptine administration increases the rates of hepatic lipogenesis *in vivo*, but has no effect on lipid synthesis in hepatocytes under the same treatment conditions. Concurrently, the administration of progesterone or bromocriptine on day 21 to the mother increases the rates of lipogenesis in the foetal liver *in vivo* on day 22. The rates of lipid synthesis in foetal isolated hepatocytes are increased by progesterone administration, but remain unchanged by bromocriptine.

INTRODUCTION

During the last 2 days of gestation the rates of lipogenesis in the liver and the plasma insulin concentrations decreased simultaneously (Lorenzo *et al.*, 1981). In addition, the rates of lipogenesis decreased between days 20 and 22 of gestation in isolated hepatocytes (Lorenzo & Benito, 1985). This factor may contribute to the fall in the hyperlipaemia found concurrently on the last day of pregnancy (Scow *et al.*, 1964). These results concur with the decrease in plasma progesterone and the peak of plasma prolactin concentration observed during the last day of gestation (Benito *et al.*, 1982*a*).

Glucocorticoid administration increased hepatic lipogenesis *in vivo* on days 20–22 by stimulating insulin secretion. The rates of lipogenesis in the liver, however, decreased during the last 2 days of gestation in glucocorticoid-treated rats as well as in the non-treated animals (Benito *et al.*, 1982*b*), even though plasma insulin concentrations remained high in the treated rats. It was observed that other hormonal changes, occurring before parturition in the rat, could be involved in the regulation of lipid synthesis in the liver (Benito *et al.*, 1982*b*).

Furthermore, the rates of lipogenesis in the foetal liver decreased at the same time as the rates in the maternal liver, during the last 2 days of gestation (Lorenzo *et al.*, 1981). However, lipogenesis in the foetal liver does not correlate with plasma insulin concentrations in the foetus (Lorenzo *et al.*, 1983). Consequently, the hormonal changes which precede parturition in the maternal circulation may control, directly or indirectly, the synthesis of fatty acids in the foetal tissues.

Accordingly, the aim of the present work was to study the effect of progesterone and bromocriptine (a well-known inhibitor of prolactin secretion) on the regulation of the rate of lipogenesis in the maternal and foetal liver, *in vivo* and in isolated hepatocytes, during the last day of gestation.

EXPERIMENTAL

Animal treatment

Pregnant Albino Wistar rats (300–350 g) fed on a stock laboratory diet (Panlab, S.L., Barcelona, Spain) were injected with bromocriptine [10 mg in 1.7 M-NaCl/ethanol (9:1, v/v) in the presence of 2 mg of tartaric acid/kg body wt.; given by Sandoz, Basle, Switzerland] (Seki *et al.*, 1974) or with progesterone [1.25 mg in olive oil:ethanol (1:1, v/v)/animal per day] (Caswell *et al.*, 1983) on day 21 of gestation. Controls were injected with 0.9% NaCl and all the carrier solutions used. Conception was assumed by the presence of spermatozoa in the vagina, and gestational age was verified by the foetal weight.

Isolation of hepatocytes

Isolated hepatocytes from maternal liver were prepared essentially by the perfusion method of Seglen (1976) in two steps: pre-perfusion with Ca^{2+}-free Krebs bicarbonate buffer containing 0.5 mM-EGTA, under O_2/CO_2 (19:1), at 37 °C for 20 min, and perfusion in the presence of 2.55 mM-Ca^{2+} and 0.5 mg of collagenase/ml under the same conditions of temperature and gassing, for 30 min. The tissue was then minced and placed in the perfusion medium in a conical flask. The mixture was incubated at 37 °C for 15 min, in a water bath under continuous gassing.

Hepatocytes from foetal rat liver were prepared by a non-perfusion collagenase dispersion method that involves incubation with Ca^{2+}-free Krebs bicarbonate buffer containing 0.5 mM-EGTA in a 150 ml conical flask for 30 min at 37 °C in a shaking water bath (100 cycles/min) under continuous gassing (O_2/CO_2, 19:1). The cell suspension was centrifuged at 50 *g* for 2 min and the medium was discarded. Cells were then resuspended in Krebs bicarbonate buffer containing 2.55 mM-Ca^{2+} and 0.5 mg of collagenase/ml in a 150 ml conical flask. The mixture was incubated at 37 °C in a shaking water bath

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(100 cycles/min) under continuous gassing. After 60 min, the cell suspension was washed with Krebs bicarbonate buffer containing 2.55 mM-Ca^{2+}, and then centrifuged at 35 *g* for 5 min and filtered through a nylon mesh (500 μm). The washing was repeated twice, with nylon mesh of 100 μm and 50 μm successively. During washings at very low speed, separation occurred between parenchymal and haematopoietic cells, the latter mostly remaining in suspension. By counting under the microscope, haematopoietic cell contamination was shown to be lower than 5%. The procedure produced approx. 1.5×10^7 cells/g of foetal liver, representing about 15% recovery. Cell viability (Trypan Blue exclusion) for both foetal and adult hepatocytes was always higher than 95%.

Determination of lipid synthesis *in vivo*

Rats on day 22 of gestation were injected intraperitoneally with 5 mCi of ^3H_2O, and 55 min later they were anaesthetized with Nembutal (50 mg/kg body wt.). Then 5 min later the abdomen was opened and maternal blood was collected from the aorta for determination of specific radioactivity of plasma water. An external-standard curve for quenching corrections was used for the calculations of radiolabelling. Foetuses were delivered by rapid hysterectomy, decapitated, exsanguinated, and foetal plasma was separated by centrifugation. Samples of maternal and foetal tissues were taken. Samples of tissue (1 g) were added to 3 ml of 30% (w/v) KOH, and the lipids were saponified and extracted by the method of Stansbie *et al.* (1976). Lipogenesis was expressed in terms of μmol of ^3H_2O incorporated into fatty acids/h per g wet wt.

Determination of lipid synthesis in isolated hepatocytes

Hepatocytes [(5–7) $\times 10^6$ cells/flask] were incubated in 25 ml Erlenmeyer flasks with Krebs–Henseleit (1932) bicarbonate buffer, pH 7.4, in a final volume of 4 ml (adult hepatocytes) or 2 ml (foetal hepatocytes) with 2.5% (w/v) fatty-acid-free bovine serum albumin in the presence of several substrates added. Cells were gassed with O_2/CO_2 (19:1) and incubated for 5 min at 37 °C in a shaking water bath (100 cycles/min). Then 0.5 mCi of ^3H_2O/flask was added to the cell suspension, and cells were incubated for a further 60 min. An external-standard curve for quenching corrections was used for measurement of radiolabelling. Lipogenesis was measured with ^3H_2O by the method described by Harris (1975), and is expressed in μmol of ^3H_2O incorporated into fatty acids/h per 10^7 cells.

Determination of hormones and metabolites

Plasma insulin was measured by radioimmunoassay by the method of Hales & Randle (1963). Plasma prolactin was measured by radioimmunoassay by using an anti-(rat prolactin) antibody obtained from N.I.H. (U.S.A.). Neutralized $HClO_4$ extracts were used to determine plasma glucose concentration (Krebs *et al.*, 1963, 1964). Plasma non-esterified 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).

RESULTS AND DISCUSSION

Rates of lipogenesis in maternal liver *in vivo* and in isolated hepatocytes on the last day of gestation

The administration of progesterone on day 21 of gestation increased the rates of lipogenesis on day 22 as compared with values for the untreated rats (Table 1). The inhibition of the rates of lipogenesis observed between days 21 and 22 of gestation in the pregnant rats (Lorenzo *et al.*, 1981) was prevented by progesterone treatment (Table 1). Concurrently, plasma glucose concentrations increased and plasma non-esterified fatty acid concentrations decreased in the treated animals (Table 1). The administration of progesterone, however, did not produce any significant change in the plasma insulin and prolactin concentrations (Table 1).

The rates of lipogenesis in isolated hepatocytes from rats on day 22 of gestation (treated with progesterone on day 21) significantly increased in the presence of endogenous substrates as compared with values for non-treated rats (Table 2). In the presence of dihydroxyacetone as substrate [which was found by Harris (1975) to be the best substrate for lipogenesis in hepatocytes from fed rats], the rates of endogenous lipogenesis increased by 85–90% in progesterone-treated and non-treated pregnant rats (Table 2). The addition of acetate (a well-known lipogenic substrate in monogastric mammals; Snoswell *et al.*, 1982) significantly increased the rates of endogenous lipogenesis in isolated hepatocytes from treated and non-treated pregnant rats by 104% and 60% respectively (Table 2). In the presence of lactate plus pyruvate (lactate being a substrate available from the foetal metabolism; Cuezva *et al.*, 1980), the rates of endogenous lipogenesis increased by 82% and 53% in isolated hepatocytes from treated and non-treated pregnant rats respectively (Table 2). Finally, after the addition of glucose as a substrate (glucose is a precursor for lipid synthesis in the liver in virgin and lactating rats; Agius & Williamson, 1980), the rates of endogenous lipogenesis did not increase significantly in isolated hepatocytes from both groups of rats studied (Table 2). This lack of effect of glucose on the rates of lipogenesis is in agreement with those results found *in vivo* in pregnant rats by Lorenzo *et al.* (1983) and Agius & Williamson (1980). However, the rates of lipogenesis in isolated hepatocytes from progesterone-treated rats were significantly higher than those from non-treated rats, regardless of the substrates added to the incubation medium (Table 2).

These results are in agreement with those obtained *in vivo*, and indicate that the stimulatory effect of progesterone on the rates of hepatic lipogenesis is due to an enhanced lipogenic capacity. Thus the fall in plasma progesterone concentration observed during the last day of gestation in the rat (Benito *et al.*, 1982*a*) may be at least partially responsible for the inhibition of the rates of lipogenesis between days 21 and 22 of gestation (Lorenzo *et al.*, 1981), probably owing to a decreased enzymic capacity. Whether the progesterone action on the rates of lipogenesis observed *in vivo* is a consequence of a direct effect or of an increase in food intake remains to be established.

The administration of bromocriptine on day 21 of gestation increased the rates of lipogenesis in the liver observed during the last day of gestation in the untreated rats (Table 1). This result is consistent with the decreased

Table 1. Effect of bromocriptine and progesterone on the rates of lipogenesis in maternal liver and on the concentration of metabolites and hormones in maternal plasma on day 22 of gestation

For details see the Experimental section. The results are means ± S.E.M. ($n = 6$–12). Rates of lipogenesis are expressed as μmol of ^3H_2O incorporated into lipid/h per g wet wt. Values that are significantly different by Student's t test from those for pregnant control rats on day 22 are shown by: $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

State of rats	Rate of lipogenesis (μmol/h per g wet wt.)	Glucose (μmol/ml)	Non-esterified fatty acids (μmol/ml)	Triacylglycerols (μmol/ml)	Insulin (μunits/ml)	Prolactin (ng/ml)
Pregnant	13.4±0.8	3.5±0.4	1.35±0.20	1.7±0.1	32±5	98±19
Pregnant, bromocriptine-treated (24 h)	17.4±0.8***	4.8±0.3*	0.73±0.10*	2.4±0.1**	23±3	37±15***
Pregnant, progesterone-treated (24 h)	16.3±0.6**	4.7±0.2**	0.53±0.08**	1.8±0.3	26±3	76±15

Table 2. Effect of bromocriptine and progesterone on the rates of lipogenesis in isolated hepatocytes from pregnant rats on day 22 of gestation

For details see the Experimental section. The results are means ± S.E.M. ($n = 4$–16), and are expressed as μmol of ^3H_2O incorporated into lipid/h per 10^7 cells. Values that are significantly different by Student's t test from those for pregnant control rats on day 22 are shown by: $^{***}P < 0.001$.

	Rate of lipogenesis				
Substrates . . . / States of rats	Endogenous substrates	Dihydroxyacetone (5 mM)	Acetate (2 mM)	Glucose (5 mM)	Lactate (10 mM) + pyruvate (1 mM)
Pregnant	0.117±0.015	0.218±0.026	0.188±0.041	0.149±0.019	0.180±0.020
Pregnant, bromocriptine-treated (24 h)	0.108±0.040	0.195±0.012	0.179±0.012	0.118±0.012	0.176±0.011
Pregnant, progesterone-treated (24 h)	0.195±0.011***	0.367±0.027***	0.398±0.050***	0.191±0.012***	0.350±0.019***

plasma non-esterified fatty acid and the increased plasma triacylglycerol concentrations observed in the treated animals (Table 1). This stimulatory effect of bromocriptine on the rates of hepatic lipogenesis is not mediated by insulin secretion, as plasma insulin concentrations remained unchanged after bromocriptine administration (Table 1). In addition, the administration of bromocriptine significantly decreased the peak of prolactin observed on day 22 of gestation in the rat (Benito *et al.*, 1982*a*; Table 1). Therefore the stimulatory effect of bromocriptine on the rate of lipogenesis is due to the fall in the plasma prolactin concentration.

On day 21 of gestation, bromocriptine administration did not change the rates of lipogenesis in isolated hepatocytes from pregnant rats on day 22, under all the conditions studied (Table 2). Thus the stimulatory effect of bromocriptine on the rates of hepatic lipogenesis observed *in vivo* (Table 1) is not due to an enhanced lipogenic capacity in isolated hepatocytes (Table 2). The peak of plasma prolactin observed during the last day of gestation in the rat (Benito *et al.*, 1982*a*) may contribute to the inhibition of the rates of hepatic lipogenesis, probably owing to decreased substrate supply to the maternal liver, as previously suggested by Agius *et al.* (1979) in lactating rats.

Rates of lipogenesis in foetal liver *in vivo* and in isolated hepatocytes on the last day of gestation

The administration of progesterone to the mother on day 21 of gestation increased the rates of lipogenesis in foetal liver on day 22 (Table 3). The fall in the rates of lipogenesis in the foetal liver during the last day of gestation in the rat (Lorenzo *et al.*, 1981) was prevented in the progesterone-treated rats (Table 3). Plasma insulin and prolactin concentrations in the foetus were not affected by progesterone administration (Table 3). Owing to the ability of maternal progesterone to cross the placenta into the foetal circulation, and to the similarity of maternal and foetal plasma progesterone concentrations (Martin *et al.*, 1977), it is reasonable to suggest a role for maternal progesterone in the control of hepatic lipogenesis in the foetuses.

Moreover, the administration of progesterone through the mother increased the rates of endogenous lipogenesis in isolated foetal hepatocytes from pregnant rats on day 22, as compared with values for non-treated rats (Table 4). In the presence of lactate plus pyruvate as substrates, the rates of endogenous lipogenesis increased by 49% and 34% in isolated foetal hepatocytes from progesterone-treated and non-treated groups of rats respectively (Table 4). When glucose was added, however, the rates of endogenous lipogenesis slightly increased in isolated hepatocytes taken from both groups of rats (Table 4). Thus lactate plus pyruvate proved to be the best substrates for foetal lipogenesis in isolated hepatocytes, even when glucose was added to lactate and pyruvate together (Table 4). The rates of lipogenesis in isolated foetal hepatocytes from progesterone-treated rats were significantly higher than those from non-treated rats, regardless of the substrates added (Table 4).

These results are in agreement with those obtained *in vivo*, and indicate that the stimulatory effect of progesterone on the rates of foetal liver lipogenesis is exerted by the hormone, owing to an enhanced lipogenic capacity. Accordingly, the fall in progesterone in the maternal circulation during the last day of gestation in the rat (Benito *et al.*, 1982*a*) may in turn decrease the enzymic capacity for lipid synthesis in the foetal liver, which results in inhibition of the rates of lipogenesis *in vivo* before birth in the rat (Lorenzo *et al.*, 1981).

The administration of bromocriptine (which crosses the placental barrier; Weinstein *et al.*, 1981) to the mother on day 21 of gestation increased the rates of lipogenesis in the foetal liver on day 22 and reached values up to those found in the control foetuses on day 21 of gestation (Lorenzo *et al.*, 1981; Table 3). Foetal plasma glucose concentrations are significantly increased in the treated animals (Table 3). Owing to the low plasma prolactin concentrations seen in the foetus on day 22 in the rat (Table 3), the inhibitory effect produced in the mother by bromocriptine (Table 1) did not seem to be involved in the observed activation of foetal liver lipogenesis after treatment (Table 3). In fact, the treatment with bromocriptine did not alter the rates of endogenous lipogenesis in isolated foetal hepatocytes from treated rats on day 22, even in the presence of other substrates added (Table 4).

Thus bromocriptine did not produce any effect on the lipogenic capacity of the foetal liver, but resulted in an increased rate of lipogenesis *in vivo*. This effect could be due to enhanced glucose availability to the foetal liver, a fact that has been considered to be the main factor involved in the regulation of foetal liver lipid synthesis in the rat (Lorenzo *et al.*, 1983).

In conclusion, our results indicate that the administration of progesterone during the last day of gestation increased the rates of lipogenesis in maternal and foetal liver as a consequence of enhanced lipogenic capacity.

Table 3. Effect of bromocriptine and progesterone on the rates of lipogenesis in foetal liver and on the concentrations of metabolites and hormones in foetal plasma on day 22 of gestation

For details see the Experimental section. The results are means ± S.E.M. (n = 6–12). Rates of lipogenesis are expressed as μmol of ^3H_2O incorporated into lipid/h per g wet wt. Values that are significantly different by Student's t test from those for pregnant control rats on day 22 are shown by: ***$P < 0.001$.

Foetuses from	Rate of lipogenesis (μmol/h per g wet wt.)	Glucose (μmol/ml)	Insulin (μunits/ml)	Prolactin (ng/ml)
Pregnant rats	9.5±0.4	2.1±0.2	90±7	10.1±0.1
Pregnant, bromocriptine-treated (24 h) rats	12.7±0.7***	4.1±0.4***	81±4	—
Pregnant, progesterone-treated (24 h) rats	13.6±0.6***	3.4±0.7**	89±8	10.1±0.1

Table 4. Effect of bromocriptine and progesterone on the rates of lipogenesis in foetal isolated hepatocytes on day 22 of gestation

For details see the Experimental section. The results are means ± S.E.M. ($n = 4$–16), and are expressed as μmol of ^3H_2O incorporated into lipid/h per 10^7 cells. Values that are significantly different by Student's t test from those for pregnant control rats on day 22 are shown by: ***$P < 0.001$.

Foetuses from	Substrates . . .	Rate of lipogenesis		
		Endogenous substrates	Lactate (10 mM) + pyruvate (1 mM)	Glucose (5 mM)	Lactate (10 mM) + pyruvate (1 mM) + glucose (5 mM)
Pregnant rats		0.079 ± 0.003	0.118 ± 0.005	0.094 ± 0.006	0.119 ± 0.005
Pregnant, bromocriptine-treated (42 h) rats		0.074 ± 0.002	0.119 ± 0.005	0.105 ± 0.003	0.127 ± 0.004
Pregnant, progesterone-treated (24 h) rats		0.126 ± 0.007***	0.169 ± 0.011***	146 ± 0.006***	0.171 ± 0.010***

The administration of bromocriptine did not change the lipogenic capacity in isolated hepatocytes from both maternal and foetal liver, but resulted in increased rates of lipogenesis *in vivo*, probably owing to an increased substrate availability for the liver. Accordingly, we suggest that the physiological decrease in progesterone and the peak of prolactin plasma concentrations at the end of the gestation seem to be at least partially responsible for the inhibition of lipogenesis *in vivo* in maternal and foetal liver during the last day of gestation in the rat. This effect is due to the inhibition of the lipogenic capacity and to decreases in substrates available for lipid synthesis.

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REFERENCES

Agius, L. & Williamson, D. H. (1980) Biochem. J. **190**, 477–480
Agius, L., Robinson, A. M., Girard, J. R. & Williamson, D. H. (1979) Biochem. J. **180**, 689–692
Benito, M., Lorenzo, M. & Medina, J. M. (1982*a*) Horm. Metab. Res. **14**, 614–615
Benito, M., Lorenzo, M. & Medina, J. M. (1982*b*) Biochem. J. **204**, 865–868
Caswell, A. M., Higham, F. C. & Bailey, E. (1983) J. Dev. Physiol. **5**, 299–305
Cuezva, J. M., Moreno, F. J., Medina, J. M. & Mayor, F. (1980) Biol. Neonate **37**, 88–95
Eggstein, M. & Kreutz, F. H. (1966) Klin. Wochenschr. **44**, 262–266
Garland, P. B. & Randle, P. J. (1962) Nature (London) **196**, 987–988
Hales, C. N. & Randle, P. J. (1963) Biochem. J. **88**, 137–146
Harris, R. A. (1975) Arch. Biochem. Biophys. **169**, 168–180
Itaya, K. & Ui, M. (1965) J. Lipid Res. **6**, 16–20
Krebs, H. A. & Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. **210**, 33–36
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. & Benito, M. (1985) Biochem. Soc. Trans. **13**, 865
Lorenzo, M., Caldés, T., Benito, M. & Medina, J. M. (1981) Biochem. J. **198**, 425–428
Lorenzo, M., Benito, M., Caldés, T. & Medina, J. M. (1983) Biochem. J. **216**, 695–699
Martin, C. E., Cake, M. H., Hartmann, P. E. & Cook, I. F. (1977) Acta Endocrinol. (Copenhagen) **84**, 157–176
Scow, R. O., Chernick, S. S. & Brinley, M. S. (1964) Am. J. Physiol. **206**, 796–804
Seglen, P. O. (1976) Methods Cell Biol. **13**, 29–83
Seki, M., Seki, K., Yoshihara, T., Watanabe, N., Okumura, T., Tajima, C., Huang, S. Y. & Kuo, C. C. (1974) Endocrinology (Baltimore) **94**, 911–914
Snoswell, A. M., Trimble, R. P., Fishlock, R. C., Storer, F. B. & Topping, D. L. (1982) Biochim. Biophys. Acta **716**, 290–297
Stansbie, D., Brownsey, R. W., Crettaz, M. & Denton, R. M. (1976) Biochem. J. **160**, 413–416
Weinstein, D., Schenker, J. G., Gloger, I., Slonim, J. H., Degroot, N., Hochbert, A. A. & Folman, R. (1981) FEBS Lett. **126**, 29–32

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