Luteal 20 α -Hydroxy Steroid Dehydrogenase and the Formation of Δ^4 -3-Oxo Steroids in the Rat after Weaning or Treatment with 2-Bromo- α -ergocryptine during Lactation

By RICHARD G. RODWAY* and NICHOLAS J. KUHN†
Department of Biochemistry, University of Birmingham, P.O. Box 363,
Birmingham B15 2TT, U.K.

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1. Natural or early weaning of rat litters caused an increased activity of maternal luteal 20 α -hydroxy steroid dehydrogenase and a decreased release of Δ^4 -3-oxo steroids *in vitro*.
2. Compound CB-154 (2-bromo- α -ergocryptine) caused an increase of 20 α -hydroxy steroid dehydrogenase activity in mid-lactation but not in early lactation.
3. Prolactin did not prevent these increases in enzyme activity.

Luteolysis in the rat is associated with increased activity of luteal 20 α -hydroxy steroid dehydrogenase and decreased activity of Δ^5 -3 β -hydroxy steroid dehydrogenase. At the same time there is a fall in the rate of secretion *in vivo*, or release *in vitro*, of Δ^4 -3-oxo steroids (Wiest *et al.*, 1968; Hashimoto *et al.*, 1968; Kuhn & Briley, 1970; Rodway & Kuhn, 1975). Although the cause of these changes has been ascribed at least partly to the withdrawal of placental lactogen or of prolactin (see Sairam & Li, 1973), the work described in the preceding paper (Rodway & Kuhn, 1975) has cast doubt on this interpretation.

Lactation is associated in the rat with functional corpora lutea and high plasma concentrations of prolactin. We have therefore studied luteal metabolism in this situation, with the hope of learning more about its regulation by prolactin.

The term 'induction' of an enzyme is used in this paper without implication of any particular mechanism.

Materials and Methods

Compound CB-154 (2-bromo- α -ergocryptine) was kindly given by Professor E. Flückiger and Dr. H. Friedli, Pharmaceutical Division, Sandoz Ltd., Basle, Switzerland. Dianil Blue 2R was from Searle Scientific Services, High Wycombe, Bucks., U.K.

To enable the corpora lutea of lactation to be distinguished from the surviving corpora lutea of pregnancy, rats were injected intraperitoneally on days 15 and 16 of pregnancy with 1 ml of 1% (w/v) Dianil Blue 2R in 0.9% (w/v) NaCl (Long & Evans, 1922). The corpora lutea of pregnancy take up and retain the dye, whereas the corpora lutea of lactation

are formed only later when the dye has disappeared from the circulation.

Other methods and materials have been described (Rodway & Kuhn, 1975).

Expression of results

Data are given as means $\pm$ S.E.M. with the numbers of rats in parentheses. S.E.M. values in Figures are shown as vertical bars.

Results

Luteal changes after weaning of the litter

Kuhn & Briley (1970) have shown that the activity of luteal 20 α -hydroxy steroid dehydrogenase acquired during parturition is lost over the first few days of lactation. Fig. 1(a) shows the reappearance of this activity at the end of normal lactation; however, the activity was due to the corpora lutea of lactation, very little being seen in those surviving from pregnancy.

Although the pups were still suckling on day 21 of lactation, they had started to eat solid food as well and it seemed probable that partial weaning had already set in. Therefore the induction might have been due to a fall in the plasma concentration of prolactin, normally maintained by the suckling stimulus (Meites & Clemens, 1972). The activity of 20 α -hydroxy steroid dehydrogenase on day 10 of lactation was greatly increased when the litter had been removed 0-3 days before (Fig. 1b). Surprisingly, this increase was mostly confined to the corpora lutea of pregnancy. This is the first demonstration that this enzyme can be repeatedly induced in the same corpora lutea, and suggests that they have retained more function than might be supposed.

Ovarian Δ^5 -3 β -hydroxy steroid dehydrogenase activity (Table 1) did not change during premature weaning, perhaps because it was still at the low con-

* Present address: Department of Reproductive Biology, University Hospital, 2105 Adelbert Road, Cleveland, Ohio 44106, U.S.A.

† To whom requests for reprints may be addressed.

Fig. 1. Changed activity of luteal 20α -hydroxy steroid dehydrogenase during lactation in the rat

(a) In corpora lutea of pregnancy (○) or of lactation (●) during undisturbed lactation. (b) On day 10 of lactation at various times after pup removal. Shaded and unshaded histogram bars indicate that proportion of the activity due to corpora lutea of pregnancy and of lactation respectively.

Table 1. Activity of Δ^5 - 3β -hydroxy steroid dehydrogenase and release of Δ^4 -3-oxo steroids after weaning

Measurements were made on day 10 of lactation after pups had been removed 0 or 3 days previously.

Time after pup removal (days)	Mean no. pups in litter	Δ^5 - 3β -Hydroxy steroid dehydrogenase activity			
		Total (nmol/min per ovary)	Corpora lutea of pregnancy (% of total)	Corpora lutea of lactation (% of total)	Remainder of ovary (% of total)
0	11.6	51.0 ± 6.2 (8)	17 (5)	62 (8)	16 (5)
3	11.0	63.7 ± 8.7 (7)	24 (2)	64 (2)	12 (2)

Time after pup removal (days)	Mean no. pups in litter	Δ^4 -3-Oxo steroid release		
		Total (nmol/h per ovary)	Corpora lutea of pregnancy (% of total)	Corpora lutea of lactation (% of total)
0	10.8	5.2 ± 0.4 (5)	36 (5)	64 (5)
3	10.5	3.1 ± 0.3 (4)	15 (4)	85 (4)

centration to which it had fallen at parturition (Kuhn & Briley, 1970), and its distribution among the different tissue elements remained constant. In contrast, the release of Δ^4 -3-oxo steroids *in vitro* was diminished by removal of the litter (Table 1). The major percentage decrease was in the corpora lutea of pregnancy, although they made the smaller contribution to the total.

The increase in activity of 20α -hydroxy steroid dehydrogenase due to litter removal was not prevented by the simultaneous injection of prolactin. Thus after 2 days the activities were 166 ± 7 (4) and 158 ± 12 (6) nmol/min per ovary with and without ovine prolactin respectively ($2 \times 400 \mu\text{g}$ daily sub-

cutaneously). After 3 days the activities were 198 ± 27 (4) and 209 ± 22 (5) nmol/min per ovary with and without rat prolactin respectively ($1 \times 200 \mu\text{g}$ daily subcutaneously).

Lactating rats were convenient to test for a possible adrenal requirement in the induction of 20α -hydroxy steroid dehydrogenase, since the foetal contribution of corticosterone makes pregnant rats unsuitable for this purpose. Three rats were bilaterally adrenalectomized at the time of litter removal on day 7 of lactation. At 2 days later the mean activity of the enzyme was 167 ± 20 nmol/min per ovary, not significantly different from the value 158 ± 12 nmol/min per ovary in unoperated rats.

Table 2. Effect of compound CB-154 during early and later lactation on activity of 20 α -hydroxy steroid dehydrogenase

During early lactation compound CB-154 was given on days 6 and 7 in 0.9% NaCl or aq. 70% (v/v) ethanol subcutaneously. During later lactation, compound CB-154 was given either on day 13 only (70%, v/v, ethanol) or on days 13 and 14 (0.9% NaCl) subcutaneously. Prolactin (2mg, NIH-P-S-11) was given on days 13 and 14 subcutaneously. Assays were carried out on day 8 or 15.

Compound CB-154	Vehicle	Mean no. of pups in litter	Mean wt. of pup stomach contents (g)	20 α -Hydroxy steroid dehydrogenase activity (nmol/min per ovary)
Day 8 of lactation				
0	None	13.8	0.5	52.5 $\pm$ 5.6 (6)
1-10	NaCl (0.9%)	11.8	0.1	40.0 $\pm$ 0.8 (5)
Day 15 of lactation				
0	NaCl (0.9%)	10.8	—	60.4 $\pm$ 6.5 (6)
0	Ethanol	12.5	0.47	63.9 $\pm$ 7.1 (6)
1	NaCl (0.9%)	12.0	0.43	286 $\pm$ 23 (4)
1	Ethanol	12.0	0.31	223 $\pm$ 30 (6)
1 plus prolactin (2mg)	Ethanol	11.8	—	237 $\pm$ 20 (4)

Fig. 2. Effect of compound CB-154 on the activity of luteal 20 α -hydroxy steroid dehydrogenase in rats during days 5-8 of pregnancy

Rats received a single initial dose of compound CB-154 (1mg in aq. 70%, v/v, ethanol subcutaneously) (●) or of aq. ethanol only (○). Others received compound CB-154 plus prolactin (2mg daily subcutaneously on days 5 and 6) (■) or compound CB-154 plus progesterone (6mg daily, subcutaneously) plus oestrone (50 μ g daily subcutaneously) (▲).

Induction of 20 α -hydroxy steroid dehydrogenase by compound CB-154

The synthetic ergot alkaloid CB-154 was used to block the release of endogenous prolactin (Flückiger, 1972; Seki *et al.*, 1974). NaCl (0.9%) suspensions given from day 6 of lactation induced no 20 α -hydroxy steroid dehydrogenase 2 days later, although milk production had greatly decreased as judged by the pup stomach weights (Table 2). However, 0.9% NaCl suspensions or aq. 70% (v/v) ethanolic solutions given on day 13 of lactation resulted in a large enzyme activity 2 days later (Table 2). This was not prevented by the simultaneous administration of

prolactin (2mg daily subcutaneously). In these litters, however, pup stomach weight fell less or not at all.

In view of these perplexing results with compound CB-154, we repeated the experiments of Lamprecht *et al.* (1969) in which the similar alkaloid ergocornine was used to induce 20 α -hydroxy steroid dehydrogenase in early pregnancy. This is a time at which the corpus luteum is believed to be under the control of pituitary prolactin (see Sairam & Li, 1973). However, compound CB-154 was retained for its apparently greater specificity (Flückiger, 1972; Seki *et al.*, 1974). Fig. 2 shows that over days 5-8 of pregnancy a single initial injection of compound CB-154 induced activities of 20 α -hydroxy steroid dehydrogenase well above the falling control values characteristic of this time (Wiest *et al.*, 1968). The activity reached 2 days after compound CB-154 administration [222 $\pm$ 37 (6) nmol/min per ovary] was decreased to 59 $\pm$ 7 (4) nmol/min per ovary by concurrent administration of prolactin (2mg daily subcutaneously), a value close to the control value [63 nmol/min per ovary (data from Rodway & Kuhn, 1975)]. Further experiments, not described in detail, showed that this induction by compound CB-154 was not reversed by simultaneously given progesterone (6mg daily subcutaneously) plus oestrone (50 μ g daily subcutaneously). No induction occurred when compound CB-154 was given on day 16 of pregnancy, by which time luteal control has largely passed to the placenta and administration of ergocornine does not interrupt pregnancy (Kirsch & Shelesnyak, 1968).

Discussion

The inhibition of prolactin secretion by ergot alkaloids and its stimulation by suckling of the young is well documented (Flückiger, 1972; Shaar & Clemens, 1972; Clemens *et al.*, 1974; Seki *et al.*,

1974; Meites & Clemens, 1972). Therefore the low activity of luteal 20α -hydroxy steroid dehydrogenase during most of lactation and its increase after weaning of the litter or administration of compound CB-154 might seem to be consistent with the concept of prolactin withdrawal as a stimulus to the induction of this enzyme (Wiest *et al.*, 1968; Lamprecht *et al.*, 1969; Kuhn, 1971; Sairam & Li, 1973). Yet this interpretation is not consistent with the failure of prolactin to prevent such induction. Nor can it explain the lack of induction by compound CB-154 early in lactation, despite the evidence that milk secretion was in fact impaired. These findings stand in contrast with the induction of 20α -hydroxy steroid dehydrogenase by compound CB-154 or ergocornine in early pregnancy, and its reversal by prolactin.

We therefore suggest that, in harmony with our experience with the late-pregnant rat (Rodway & Kuhn, 1975), circulating prolactin does not regulate the activity of this luteal enzyme during these times. This conclusion agrees with the failure of prolactin antiserum to increase the secretion of 20α -hydroxypregn-4-en-3-one from the ovary of the lactating rat (Yoshinaga, 1974). Weaning is known to raise the concentration of plasma luteotrophin (luteinizing hormone) (Ford & Melampy, 1973), whereas human chorionic gonadotrophin (which resembles luteotrophin in its biological activity) can induce 20α -hydroxy steroid dehydrogenase during lactation (Rodway & Kuhn, 1975). So luteotrophin could possibly account for the enzyme induction after weaning, although it may not be responsible for that seen after compound CB-154, which appears not to increase plasma concentrations of this hormone (Seki *et al.*, 1974).

It has been suggested that increased activity of 20α -hydroxysteroid dehydrogenase, decreased activity of Δ^5 - 3β -hydroxy steroid dehydrogenase and decreased release of Δ^4 -3-oxo steroids by luteal tissue *in vitro* together form part of a multiple response pattern characteristic of luteolysis in the rat (Rodway & Kuhn, 1975). The fall in the rate of Δ^4 -3-oxo steroid release seen after weaning is in line with this proposal.

The unchanged activity of Δ^5 - 3β -hydroxy steroid dehydrogenase, still low after its fall at parturition, implies that these three factors may not all return to the 'pre-luteolytic' condition at the same rate.

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