Hormonal Control of Luteal 20 α -Hydroxy Steroid Dehydrogenase and Δ^5 -3 β -Hydroxy Steroid Dehydrogenase during Luteolysis in the Pregnant Rat

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1. Treatment of pregnant rats with human chorionic gonadotrophin, luteotrophin (luteinizing hormone), luteotrophin-releasing hormone, prostaglandin F_{2 α} , aminoglutethimide, or by foetoplacental removal or hysterectomy achieved a common multiple-response pattern, namely increased activity of luteal 20 α -hydroxy steroid dehydrogenase with decreased activity of Δ^5 -3 β -hydroxy steroid dehydrogenase and release of Δ^4 -3-oxo steroids *in vitro*. 2. Similar effects of foetoplacental removal were noted in pregnant mice. 3. Gonadotrophin induced lower activities of 20 α -hydroxy steroid dehydrogenase, except at the very end of pregnancy, and partly inhibited the induction caused by foetoplacental removal. 4. The results suggest the existence of a placental factor that restrains these changes until the end of normal pregnancy, which is produced in amounts proportional to the number of placentae and is conveyed to the ovary via the blood. 5. This factor was not replaced by prolactin. 6. It is argued that neither placental lactogen nor pituitary luteotrophin participate in the induction of 20 α -hydroxy steroid dehydrogenase at late pregnancy in the rat. 7. Aminoglutethimide induced 20 α -hydroxy steroid dehydrogenase only in late pregnancy. This was partly reversed by progesterone, wholly reversed by progesterone plus oestrogen, and did not involve the pituitary.

The induction of 20 α -hydroxy steroid dehydrogenase is a striking feature of luteolysis in the late pregnant rat, and is largely responsible for the withdrawal of circulating progesterone that precedes parturition and lactogenesis (Wiest *et al.*, 1968; Kuhn & Briley, 1970). The cause of this induction has been ascribed to the surge of luteotrophin (luteinizing hormone) that occurs at parturition, and to the presumed withdrawal of placental lactogen (Lamprecht *et al.*, 1969; Kuhn, 1971; Sairam & Li, 1973). These conclusions are supported by the induction of 20 α -hydroxy steroid dehydrogenase during pregnancy either after treatment with luteotrophin or gonadotrophin (human chorionic), or after removal of the placentae or the entire gravid uterus. The induction due to the latter operation has been reversed by prolactin (Wiest *et al.*, 1968). Further, the enzyme can be induced in early pregnancy by ergocornine, an agent that inhibits secretion of pituitary prolactin, and this is also reversed by exogenous prolactin (Lamprecht *et al.*, 1969).

It appeared to us, however, that the above scheme did not accord with the known timing of luteotrophin and prolactin surges around parturition, nor with the induction of 20 α -hydroxy steroid dehydrogenase in

hypophysectomized late-pregnant rats (Wiest *et al.*, 1968). We now report our findings on changes in luteal 20 α -hydroxy steroid dehydrogenase and Δ^5 -3 β -hydroxy steroid dehydrogenase activity and in the release of Δ^4 -3-oxo steroids, during pregnancy of the rat and mouse. It is suggested that these, and other, changes constitute a multiple-response pattern to hormonal stimuli not accounted for by prolactin or luteotrophin.

For the sake of brevity the term 'induction' is used without any implication of a particular mechanism.

Experimental

Materials

The following were generously donated: ovine prolactin (NIH-P-S-10 and NIH-P-S-11), rat prolactin (NIH-RP1) and ovine luteotrophin (NIH-LH-18) from the Pituitary Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md., U.S.A.; prostaglandin F_{2 α} (tromethamine salt) from Dr. J. E. Pike, The Upjohn Co., Kalamazoo, Mich., U.S.A.; anti-oestrogen compound CN-55945-27 from Parke Davis Ltd., Newport, Mon., U.K.; aminoglutethimide from Dr. D. M. Burley, CIBA Laboratories, Horsham, Sussex RH12 4AB, U.K.

Bovine serum albumin (Cohn fraction V), gonadotrophin and steroids were from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Enzymes

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and nucleotides were from The Boehringer Corp. (London) Ltd., London W.5, U.K. Fluothane was from I.C.I. Ltd., Macclesfield, Cheshire, U.K.

Hypophysectomized timed-pregnant rats of Wistar strain were purchased from Carworth Europe, Alconbury, Huntingdon, U.K. All other rats were bred from a Wistar-derived stock maintained in this Department and were in their first pregnancy. Weights were approximately 330 g in late pregnancy. Mice of strain CD1, category 4, were from Glaxo Research Farm, Middx., U.K. All animals were maintained with food and water *ad lib.* under a 14h-light–10h-dark cycle. During mating, rats were checked each morning for the appearance of a vaginal plug. Day 1 of pregnancy refers to the day on which a plug was first found, after which rats littered on day 22–23.

Expression of results

Results are given in the legends and in the text as means $\pm$ S.E.M., with the numbers of animals shown in parentheses. In Figures, S.E.M. are shown as vertical bars. Where required, statistical significance was calculated by the Student's *t* test.

Methods

20 α -Hydroxy steroid dehydrogenase was assayed at pH 10, and Δ^5 -3 β -hydroxy steroid dehydrogenase at pH 9.4 (Kuhn & Briley, 1970) and activities are expressed as nmol/min per ovary at 37°C at these pH values. Δ^4 -3-Oxo steroids were determined by the method of Flint & Denton (1969) with application of the Allen (1950) correction. Lactose was extracted from mammary tissue as described by Kuhn & Lowenstein (1967) and determined enzymically by the method of Reithel (1965).

Cholesterol and cholesterol ester were extracted from the corpora lutea (Folch *et al.*, 1957) and separated by t.l.c. on silica gel G, with chloroform–cyclohexane (1:1, v/v). Eluted cholesterol and ester were determined by the Lieberman–Burchard method (Varley, 1958).

Hormones were administered in a variety of ways, as described in the legends to Figures and Tables. Suspensions of steroids in arachis oil were warmed at 37°C for 1 day before injection. Aminoglutethimide and luteotrophin-releasing hormone were infused in 0.9% (w/v) NaCl or 4.5% (w/v) glucose to which benzylpenicillin (600 i.u./ml) and streptomycin sulphate (200 i.u./ml) were added. A peristaltic pump (LKB Varioperspex) was used to infuse this solution into the cannulated external jugular vein at about 0.25 ml/h.

Placental homogenates were prepared in sufficient 0.9% (w/v) NaCl to give a suspension able to be injected. Extracts were prepared by homogenizing equal volumes of placenta and 0.9% (w/v) NaCl

followed by centrifugation at 150g for 10 min. The clear supernatant was injected.

Hysterectomy and foetoplacental removal was performed by ventral laparotomy. Cannulation of the external jugular vein was performed by the method of Popovic & Popovic (1960). Operations were done under anaesthesia with diethyl ether, Nembutal or halothane–O₂ as convenient. Corpora lutea were separated from remaining ovarian tissue, in chilled 0.9% (w/v) NaCl, with the aid of a dissection needle and fine forceps.

Results

Ovarian changes after administration of gonadotrophin

A dose–response curve showed that a maximum induction of 20 α -hydroxy steroid dehydrogenase at 24 h was given by 100 i.u. of gonadotrophin (Fig. 1). In these experiments a 24 h response was chosen to lie on the rising portion of the time curve (see Fig. 2), and in subsequent work at least 100 i.u. of gonadotrophin was used to ensure a maximum response.

The temporal rise in activity of 20 α -hydroxy steroid dehydrogenase showed a small lag and reached a plateau of about 100 nmol/min per ovary at 3 days (Fig. 2), confirming the pattern found by Kuhn (1972). At the same time there was a reciprocal fall in the release of Δ^4 -3-oxo steroids by incubated luteal tissue (Fig. 2) and in the activity of Δ^5 -3 β -hydroxy steroid dehydrogenase (Fig. 3). The tendency of both to rise again on day 4 is of secondary interest and may be due to the multiple ovulation and stimulation of new follicles by the prolonged treatment with gonadotrophin. Thus the increased activity of Δ^5 -3 β -hydroxy steroid dehydrogenase was entirely

Fig. 1. Induction of luteal 20 α -hydroxy steroid dehydrogenase in rats by different doses of gonadotrophin

Assays were carried out on day 17 of pregnancy, 24 h after a single subcutaneous dose of gonadotrophin in 0.9% NaCl.

due to elements of the ovary other than the corpora lutea of pregnancy, which themselves showed a marked decrease (Fig. 3). In a separate experiment (results not shown) aminogluthethimide (0.2mg/ml) in the incubation medium completely blocked the release of Δ^4 -3-oxo steroids. Since this agent is known to inhibit cholesterol side-chain cleavage, it was taken as evidence for the true synthesis of these steroids rather than for their simple release from some store.

The corpora lutea were more responsive to gonadotrophin at times very close to parturition. Thus in a separate experiment treatment of rats with gonadotrophin (250i.u. daily) for 2 days starting on day 16 or 19 of pregnancy induced 20 α -hydroxy steroid dehydrogenase activities of 125 ± 11 (9) and 198 ± 17 (8) nmol/min per ovary on days 18 and 21 respectively ($P < 0.01$). No enzyme is normally evident on the morning of day 21.

In view of the findings in Fig. 2, and of the fact that a partial fall in the secretion of Δ^4 -3-oxo steroids from the ovary precedes the induction of 20 α -hydroxy steroid dehydrogenase in late pregnancy (Fajer & Barraclough, 1967; Hashimoto *et al.*, 1968), several steroids were tested for their possible ability to prevent the induction of 20 α -hydroxy steroid dehydrogenase by gonadotrophin. Table 1 (Expt. A) shows that a statistically significant inhibition was given by single daily doses of 12mg of progesterone ($P < 0.02$), but not by twice daily doses of 6mg, nor by pregnenolone or pregnanedione. When progesterone was tested on rats receiving sub-optimum doses of gonadotrophin, possibly a more sensitive system, no inhibition was found (Table 1, Expt. B). These results do not convincingly support

an inhibitory role for progesterone. On a later occasion progesterone (6mg) plus oestrone (50 μ g) were tested, but also proved ineffective. These results contrast with the ability of progesterone and oestrogen to inhibit the induction of this enzyme by aminogluthethimide (see below).

Corpora lutea were assayed for cholesterol and its ester to see if the known 'cholesterol-depleting' ability of gonadotrophin (Claesson & Hillarp, 1947; Bell *et al.*, 1964) could account for the decreased rate

Fig. 2. Increased activity of luteal 20 α -hydroxy steroid dehydrogenase (●) and decrease of Δ^4 -3-oxo steroid release (○) *in vitro* in rats after administration of gonadotrophin

Assays were performed on day 20 of pregnancy after daily subcutaneous administration of gonadotrophin (10i.u.) for 0–3 days.

Fig. 3. Changes in activity of Δ^4 -3 β -hydroxy steroid dehydrogenase in rats after gonadotrophin (●) or foetoplacental removal (○)

Assays were performed on day 20 of pregnancy at various times after foetoplacental removal or daily subcutaneous doses of gonadotrophin (100i.u.). Histograms show the enzyme activity due to corpora lutea of pregnancy (shaded) and of remaining tissue (unshaded).

Table 1. Effect of injected steroids on the activity of luteal 20 α -hydroxy steroid dehydrogenase after administration of gonadotrophin

Expt. A, gonadotrophin (250i.u.) injected daily for 3 days. Expt. B, gonadotrophin (50i.u.) given on 1 day. Assays were carried out 24h after the last dose. Steroids were given subcutaneously, once or twice daily at the dose shown.

Steroid	20 α -Hydroxy steroid dehydrogenase activity (nmol/min per ovary)
Expt. A	
None	96.3 $\pm$ 6.5 (5)
Progesterone (1 $\times$ 12mg)	65.4 $\pm$ 7.2 (5)
Progesterone (2 $\times$ 6mg)	118 $\pm$ 7 (5)
Pregnenolone (1 $\times$ 12mg)	105 $\pm$ 9 (5)
5 α -Pregnanedione (1 $\times$ 12mg)	106 $\pm$ 12 (4)
Expt. B	
None	33.5 $\pm$ 7.4 (5)
Progesterone (1 $\times$ 6mg)	32.5 $\pm$ 5.4 (4)
Progesterone (1 $\times$ 12mg)	30.1 $\pm$ 3.8 (5)

Fig. 4. Changes in the cholesterol (○) and cholesterol ester (●) content of corpora lutea of pregnancy in rats after gonadotrophin

Assays were performed on day 20 of pregnancy at various times after starting treatment with gonadotrophin (100 i.u. subcutaneously injected daily).

of Δ^4 -3-oxo steroid release. After treatment with gonadotrophin for 3 days there was a marked fall in cholesterol ester, but little or none in free cholesterol (Fig. 4). Therefore the decreased release of Δ^4 -3-oxo steroids may not be due to a shortage of precursor cholesterol, as proposed by Armstrong *et al.* (1964), unless transport into the mitochondria is limiting.

Luteal 20α -hydroxy steroid dehydrogenase could also be induced during lactation. Gonadotrophin (100 i.u.) given daily on days 7, 8 and 9 of lactation resulted in a mean activity of 146 ± 6 (4) nmol/min per ovary on day 10. This compares with a mean activity of 53 ± 16 (6) nmol/min per ovary in untreated rats at this stage.

Induction of 20α -hydroxy steroid dehydrogenase by luteotrophin and luteotrophin-releasing hormone

As gonadotrophin is specifically a primate hormone, the magnitude of enzyme induction by luteotrophin was tested. In one group of rats ovine luteotrophin (subcutaneous injection of 100 or 200 μ g in oil daily) induced a mean 20α -hydroxy steroid dehydrogenase activity of 147 ± 12 (5) nmol/min per ovary after 2 days, assayed on day 21 of pregnancy.

Luteotrophin-releasing hormone infused over 2 days was also able to induce this enzyme, though apparently only at late pregnancy. Thus infusion of 0, 100 or 500 ng/h on days 19–21 resulted in mean activities of 13.8 (2), 67.4 ± 18 (6) and 205 ± 20 (5) nmol/min per ovary respectively. Infusion of 100 ng/h on days 16–18 gave a mean activity of 20.2 (2) nmol/min per ovary. Therefore both endogenously and exogenously supplied luteotrophin can induce this enzyme near parturition.

Fig. 5. Increased activity of luteal 20α -hydroxy steroid dehydrogenase in rats after foetoplacental removal (●) or hysterectomy (▲), and decreased secretion of Δ^4 -3-oxo steroids (○) after foetoplacental removal

Assays were performed on day 20 of pregnancy at various times after operation.

Luteal changes after foetoplacental removal in the rat

A rise in the activity of 20α -hydroxysteroid dehydrogenase (Fig. 5) and a fall in the activity of Δ^5 - 3β -hydroxy steroid dehydrogenase (Fig. 3) and in the release of luteal Δ^4 -3-oxo steroids (Fig. 5) occurred on day 20 of pregnancy after fetuses and placentae had been removed at various times beforehand. These changes closely resembled those seen after treatment with gonadotrophin except that 20α -hydroxy steroid dehydrogenase rose to about twice the activity. When these measurements were repeated about 9 months later, much larger responses to foetoplacental removal were seen, reaching around 317 ± 16 (14) nmol/min per ovary, though there was no increased response to gonadotrophin. This change was not correlated with the time of year and its causes remain obscure. The secondary increase in activity of Δ^5 - 3β -hydroxy steroid dehydrogenase that was seen after gonadotrophin treatment was not evident after foetoplacental removal; nor was there any sign of ovulation. The decrease in activity of this enzyme after 4 days was entirely accounted for by the corpora lutea of pregnancy (Fig. 3).

The activity of 20α -hydroxy steroid dehydrogenase that was normally attained 2 days after complete foetoplacental removal [166 ± 16 (6) nmol/min per ovary] was decreased to 70 ± 24 (5) when one foetoplacental unit was left intact and to 22 ± 13 (4) nmol/min per ovary when two were left intact. When two foetoplacental units remained in one uterine horn,

adjacent to the ovary, equally little induction of 20α -hydroxy steroid dehydrogenase occurred in either ovary [ipsilateral, 26 ± 11 (3) nmol/min per ovary; contralateral, 20 ± 5 (3) nmol/min per ovary].

Attempts were made with injected prolactin or placental extracts to inhibit the induction that followed foetoplacental removal. No significant inhibition was shown by bovine or rat prolactin administered subcutaneously at doses of 0.2 or 1 mg/day. Placental extracts or homogenates, administered intraperitoneally or subcutaneously at doses ranging from 6 to 40 placental equivalents daily over 1–2 days, also gave no evident inhibition (results not shown). The placentae were derived from donor rats at various stages of pregnancy, including days 11–13 when placental lactogen appears to be most abundant (Ray *et al.*, 1955; Mathies, 1967; Linkie & Niswender, 1971, 1973). In other experiments progesterone, oestrone, oestradiol- 17β , oestriol, progesterone plus oestrone, and the anti-oestrogen compound CN-55945-27 were unable to inhibit the induction of 20α -hydroxy steroid dehydrogenase. However, gonadotrophin (250 i.u. daily) inhibited 49% [without gonadotrophin, 317 ± 16 (14) nmol/min per ovary; plus gonadotrophin, 162 ± 8 (6) nmol/min per ovary].

Induction of 20α -hydroxy steroid dehydrogenase after foetoplacental removal in the mouse

In view of the failure to demonstrate directly an inhibitory component of rat placentae, the mouse was tested as a possibly more amenable recipient. It was

first necessary to establish the exact timing of the normal rise in activity of 20α -hydroxy steroid dehydrogenase around parturition. Fig. 6(a), which includes the data of Owen & Kuhn (1971), shows that the enzyme appeared after day 18 of pregnancy (parturition occurred on day 19–20) and continued to rise in activity until at least day 4–5 of lactation. Thus its appearance is similar to, though slower than, that in the rat. Fig. 6(b) shows the enzyme activity on day 17 of pregnancy after foetoplacental removal at various times beforehand. The response is also similar to that in the rat, though slower. Again, however, it was not possible to prevent this response by injection of rat placental extracts over 3 days [without extracts, 50 ± 10 (5) nmol/min per ovary; plus extracts, 56 ± 13 (6) nmol/min per ovary].

Induction of 20α -hydroxy steroid dehydrogenase after hysterectomy

Our inability to inhibit the induction of 20α -hydroxy steroid dehydrogenase after foetoplacental removal might have been due to the overriding action of some uterine luteolysin. Evidence for such a substance in the rat exists (Anderson *et al.*, 1966; Barley *et al.*, 1966; Clemens *et al.*, 1968). Moreover in the experiments of Wiest *et al.* (1968) where small doses of prolactin were highly inhibitory, hysterectomized rats were used. Fig. 5 shows the appearance of 20α -hydroxy steroid dehydrogenase activity in our rats after hysterectomy on day 17 of pregnancy. The time-course is very similar to that seen after foetoplacental removal or treatment with gonadotrophin. In

Fig. 6. Increased activity of luteal 20α -hydroxy steroid dehydrogenase in mice

(a) At approx. normal parturition as measured by us (●) or by Owen & Kuhn (1971) (○). (b) On day 17 of pregnancy at various times after foetoplacental removal.

particular there is no sign of the lag that might be expected if a luteolytic, and therefore inductive, substance were being removed. In further experiments the simultaneous administration of ovine prolactin (0.4–2.5 mg daily subcutaneously) gave no consistent inhibition [pooled results: plus prolactin, 165 ± 29 (9) nmol/min per ovary; without prolactin, 204 ± 25 (8) nmol/min per ovary].

The possibility remained that the ovarian refractoriness to prolactin was due to the presence of the pituitary, as suggested by Malven *et al.* (1967), since Wiest *et al.* (1968) had used hypophysectomized rats. When a small number of hypophysectomized rats were tested, however, prolactin (2 mg daily subcutaneously) did not inhibit the activity of 20α -hydroxy steroid dehydrogenase activity due to hysterectomy (plus prolactin, 429 nmol/min per ovary; without prolactin, 406 nmol/min per ovary; each a mean of two rats. N.B. The unusually high activities in these rats probably reflects their different source).

Luteal changes after administration of prostaglandin $F_{2\alpha}$

In view of the known luteolytic effects of prostaglandin $F_{2\alpha}$, especially its ability to increase the 20α -hydroxypregn-4-en-3-one/progesterone ratio in ovarian secretions (Pharris & Wyngarden, 1969; Kirton *et al.*, 1970; Behrman *et al.*, 1971), we tested its effect on enzyme concentrations in the ovaries of pregnant rats. Solutions of the tromethamine salt were injected subcutaneously three times daily at different stages of pregnancy. 20α -Hydroxy steroid dehydrogenase was induced slowly, and to a small extent in the middle of pregnancy, but more extensively and rapidly as parturition was approached (Fig. 7). Since these experiments were completed, similar findings have been reported by Strauss & Stambaugh (1974) and by Fuchs & Mok (1974). In a separate experiment (Table 2) prostaglandin $F_{2\alpha}$ caused an increase in the activity of 20α -hydroxy steroid dehydrogenase that was not prevented by progesterone plus oestrone. It also led to a fall in the activity of Δ^5 - 3β -hydroxy steroid dehydrogenase and to the appearance of mammary lactose, this latter effect confirming the observation of Deis (1971) on milk accumulation and presumably reflecting a withdrawal of ovarian progesterone.

Induction of 20α -hydroxy steroid dehydrogenase by aminoglutethimide

Administration of aminoglutethimide (25 mg) twice daily on days 18 and 19 or 19 and 20 of pregnancy resulted in high concentration of luteal 20α -hydroxy steroid dehydrogenase on day 20 or 21 respectively (Table 3). The later treatment appeared

to be more effective. Still later administration was not attempted since the enzyme activity normally starts to rise spontaneously on day 21–22 (Wiest *et al.*, 1968; Kuhn & Briley, 1970). In some subsequent experiments the enzyme was as a routine induced by a single initial dose of aminoglutethimide (25 mg), which was found to be equally effective. Although plasma concentrations of Δ^4 -3-oxo steroids were not determined, the appearance of mammary lactose (Table 3) was indicative of the interruption of progesterone secretion, since it is known that lactogenesis in the rat is specifically under the restraint of this hormone (Kuhn, 1969a,b).

When aminoglutethimide was given before day 18 of pregnancy there was no induction of 20α -hydroxy steroid dehydrogenase at all. Since the drug might have been less effective in the face of the higher rates of Δ^4 -3-oxo steroid secretion that occur at this stage of pregnancy, two rats were hemi-ovariectomized in addition to receiving aminoglutethimide on days 16 and 17. On day 18, however, little 20α -hydroxy steroid dehydrogenase activity was seen (mean = 2.8 nmol/min per ovary), although milk was visible in the mammary glands. Hemi-ovariectomy alone did not cause milk secretion.

In three rats an initial injection of aminoglutethimide (10 mg) on day 16 of pregnancy was followed by continuous infusion of 100 mg during the next 2 days. The subsequent mean enzyme activity was only 6.5 ± 0.5 nmol/min per ovary. Aminoglutethimide (25 mg) injected twice daily on days 13 and 14 of lactation resulted in a mean enzyme activity on day 15 of 59 ± 17 (3) nmol/min per ovary, indistinguishable from the value 60 ± 7 (6) nmol/min per ovary in control animals.

Time-course studies revealed a lag of at least 1 day before the enzyme activity rose after aminoglutethimide.

Fig. 7. Induction of luteal 20α -hydroxy steroid dehydrogenase in pregnant rats by prostaglandin $F_{2\alpha}$

Rats received 0.75 mg of prostaglandin $F_{2\alpha}$ (tromethamine salt) daily in three equal subcutaneous doses, on each day of treatment.

Table 2. *Change in 20 α -hydroxy steroid dehydrogenase, Δ^5 -3 β -hydroxy steroid dehydrogenase and mammary lactose after administration of prostaglandin F_{2 α}*

Prostaglandin F_{2 α} was given subcutaneously in three equal doses on each of days 16 and 17 of pregnancy. Progesterone and oestrone were given subcutaneously once daily. Assays were carried out on day 18.

Dose of prostaglandin F _{2α} (mg/day)	20 α -Hydroxy steroid dehydrogenase activity (nmol/min per ovary)	Δ^5 -3 β -Hydroxy steroid dehydrogenase activity (nmol/min per ovary)	Mammary lactose (μ mol/g of tissue)
0	4.1 $\pm$ 1.7 (22)	90.9 $\pm$ 5.3 (11)	0.3 (2)
0.75	172 $\pm$ 8 (4)	24.3 $\pm$ 4.0 (4)	8.7 $\pm$ 0.7 (4)
1.25	158 $\pm$ 22 (4)	34.4 $\pm$ 5.0 (4)	12.2 (2)
0.75 plus progesterone (6mg) plus oestrone (50 μ g)	213 (2)	—	—

Table 3. *Aminogluthethimide induction of luteal 20 α -hydroxy steroid dehydrogenase and mammary lactose*

Aminogluthethimide (25 mg) was injected subcutaneously twice daily. Assays were performed 2 days after the first injection.

Material injected	Period of injection (day of pregnancy)	Time of assay (day of pregnancy)	20 α -Hydroxy steroid dehydrogenase activity (nmol/min per ovary)	Mammary lactose (μ mol/g of tissue)
Aminogluthethimide	15 and 16	17	—	7.2 (2)
Aminogluthethimide	16 and 17	18	7.5 $\pm$ 4.9 (3)	—
Aminogluthethimide	18 and 19	20	135 $\pm$ 28 (3)	—
Aminogluthethimide	19 and 20	21	220 $\pm$ 15 (12)	5.9 $\pm$ 1.1 (3)
NaCl (0.9%) only	19 and 20	21	5.3 $\pm$ 3.4 (6)	0.3 (2)

ethimide treatment. Thus after treatment was begun on day 19 of pregnancy, no activity was present on day 20 [0.0 (2) nmol/min per ovary]. Likewise, treatment on day 20 resulted in negligible activity on day 21 [4.7 $\pm$ 3.4 (3) nmol/min per ovary].

Inhibition by progesterone and oestrogen of induction of 20 α -hydroxy steroid dehydrogenase by aminogluthethimide

Several steroids were tested for their possible ability to prevent the induction of 20 α -hydroxy steroid dehydrogenase (Table 4). Progesterone oil and 0.9% NaCl suspensions were effective intraperitoneally but not subcutaneously, but no greater inhibition was given by larger doses. A slight apparent inhibition of 35% ($P < 0.2$) was shown by pregnenolone, but none by 17 α -hydroxyprogesterone, 5 α -pregnanedione, or 11 β -hydroxyprogesterone. In a separate series of rats the synthetic progestin 7 α -methoxyprogesterone (medroxyprogesterone) (6mg daily in oil injected intraperitoneally) did not prevent the induction of enzyme by aminogluthethimide.

Oestrone and oestradiol-17 β almost completely inhibited enzyme induction at a daily dose of 50 μ g injected intraperitoneally, and substantially inhibited at daily doses of 5 μ g injected intraperitoneally or subcutaneously. Oestriol was less effective. It was necessary to administer the oestrogens together

with subcutaneous progesterone to prevent their abortifacient activity, since placental removal itself induces 20 α -hydroxy steroid dehydrogenase activity (Wiest *et al.*, 1968; the present paper, Fig. 5). This abortifacient activity may account for the higher enzyme activities seen in rats that received very large doses of oestrogen (Table 4, Expt. C).

Further experiments were designed to see whether the induction of 20 α -hydroxy steroid dehydrogenase or its inhibition by oestrogen involved the pituitary. The possibility existed that the induction was really due to luteotrophin released in response to changes in circulating steroids. Moreover the inhibition by oestrogen might have been secondary to a burst of prolactin (Niswender *et al.*, 1969; Chen & Meites, 1970), known to block the induction of 20 α -hydroxy steroid dehydrogenase under some circumstances (Wiest *et al.*, 1968; Lamprecht *et al.*, 1969). The first possibility was ruled out by the observation that induction by aminogluthethimide was effective in hypophysectomized rats [plus aminogluthethimide, 286 $\pm$ 38 (6) nmol/min per ovary; without aminogluthethimide, 12.7 (2) nmol/min per ovary]. Two of the hypophysectomized rats aborted during the night before being killed. Although placental removal at this late stage was unlikely to have induced high concentrations of enzyme, the remaining rats were given progesterone (3mg on day 1, 6 mg on day 2, injected subcutaneously) to prevent abortion. The

Table 4. *The ability of various steroids to inhibit the induction of luteal 20 α -hydroxy steroid dehydrogenase by aminogluthethimide*

All rats received aminogluthethimide (25 mg) twice daily on days 19 and 20 of pregnancy, and were killed for assay on day 21. Rats in Expts. B and C were injected subcutaneously or intraperitoneally respectively with progesterone (6 mg) once daily in addition to the steroids listed, which were also given once daily unless otherwise shown.

	20 α -Hydroxy steroid dehydrogenase (nmol/min per ovary)
Expt. A	
None	220 $\pm$ 15 (12)
Corticosterone (6 mg, subcutaneous)	227 (2)
Progesterone (6 mg, subcutaneous)	189 $\pm$ 7 (5)
Progesterone (6 mg, intraperitoneal)	109 $\pm$ 24 (6)
Progesterone (2 $\times$ 30 mg, intraperitoneal)	111 $\pm$ 33 (7)
Pregnenolone (2 $\times$ 30 mg, intraperitoneal)	143 $\pm$ 27 (6)
17 α -Hydroxyprogesterone (2 $\times$ 30 mg, intraperitoneal)	264 $\pm$ 29 (4)
Expt. B	
None	189 $\pm$ 7 (5)
Progesterone (6 mg, intraperitoneal)	81 $\pm$ 28 (3)
Pregnenolone (6 mg, intraperitoneal)	156 $\pm$ 5 (3)
Pregnanedione (6 mg, intraperitoneal)	184 $\pm$ 10 (3)
17 α -Hydroxyprogesterone (6 mg, intraperitoneal)	175 $\pm$ 12 (3)
11 β -Hydroxyprogesterone (6 mg, intraperitoneal)	159 $\pm$ 12 (3)
Oestriol (50 μ g, intraperitoneal)	102 $\pm$ 51 (3)
Oestradiol-17 β (50 μ g, intraperitoneal)	13 $\pm$ 0.3 (3)
Oestrone (50 μ g, intraperitoneal)	4.1 $\pm$ 2.7 (5)
Oestrone (5 μ g, intraperitoneal)	36.5 $\pm$ 7.2 (4)
Oestrone (5 μ g, subcutaneous)	42.2 $\pm$ 6.9 (4)
Expt. C	
None	109 $\pm$ 24 (6)
5 α -Pregnane-3,20-dione (2 $\times$ 30 mg, intraperitoneal)	83 $\pm$ 36 (4)
11 β -Hydroxyprogesterone (2 $\times$ 30 mg, intraperitoneal)	174 $\pm$ 37 (4)
17 α -Hydroxyprogesterone (2 $\times$ 30 mg, intraperitoneal)	111 $\pm$ 33 (4)
Oestriol (2 $\times$ 50 μ g, intraperitoneal)	151 (2)
Oestradiol-17 β (2 $\times$ 50 μ g, intraperitoneal)	95 $\pm$ 20 (4)
Oestrone (2 $\times$ 50 μ g, intraperitoneal)	38.6 $\pm$ 6.5 (3)

above results have been pooled. That oestrogen was not acting via prolactin was shown in two ways. First, the additional injection of compound CB-154 (2-bromo- α -ergocryptine) to block prolactin release (Flückiger, 1972; Karg *et al.*, 1972; Seki *et al.*, 1974) did not reverse its inhibition of enzyme induction [plus oestrone plus compound CB-154, 18 $\pm$ 5 (5) nmol/min per ovary; plus oestrone without compound CB-154, 24 $\pm$ 12 (4) nmol/min per ovary; without either, 263 $\pm$ 28 (11) nmol/min per ovary]. Secondly, ovine prolactin itself (subcutaneous injection of 2 mg daily) did not inhibit induction due to aminogluthethimide [plus prolactin, 234 $\pm$ 18 (3) nmol/min per ovary; without prolactin, 263 $\pm$ 28 (11) nmol/min per ovary].

Decrease in activity of Δ^5 -3 β -hydroxy steroid dehydrogenase after aminogluthethimide

In a small number of normal or hypophysectomized rats aminogluthethimide treatment (25 mg) on day 19 of pregnancy resulted in a decreased activity of

Δ^5 -3 β -hydroxy steroid dehydrogenase on day 21. The pooled values are: 0.9% NaCl controls, 60 $\pm$ 3.4 (5) nmol/min per ovary; aminogluthethimide-treated, 38 $\pm$ 2.5 (5) nmol/min per ovary.

Discussion

A feature of luteolysis in the late-pregnant rat is the partial decline in ovarian secretion of Δ^4 -3-oxo steroids that precedes the induction of 20 α -hydroxy steroid dehydrogenase (Fajer & Barraclough, 1967; Hashimoto *et al.*, 1968). Since the injected gonadotrophin or luteotrophin which induces this enzyme during pregnancy similarly impairs the ability of luteal tissue to release Δ^4 -3-oxo steroids *in vitro* (Armstrong *et al.*, 1964), some of our experiments were designed to test a possible cause-and-effect relationship between these two parameters. Δ^5 -3 β -Hydroxy steroid dehydrogenase was also assayed because its activity in the ovary falls at parturition (Kuhn & Briley, 1970) and because it might catalyse a rate-limiting step on the pathway of Δ^4 -3-oxo

steroid synthesis. This latter possibility has grown since the recent upward revision of rates of progesterone production in late pregnancy (Pepe & Rothchild, 1974).

Treatment of pregnant rats with gonadotrophin or prostaglandin $F_{2\alpha}$ and the removal of fetuses and placentae or of the whole gravid uterus brought about in each case an increase in activity of 20α -hydroxy steroid dehydrogenase with a decrease in that of Δ^5 - 3β -hydroxy steroid dehydrogenase and in the release of Δ^4 -3-oxo steroids *in vitro*; but there was no sign that one response preceded the other. Further, injected steroids were unable to prevent the induction of 20α -hydroxy steroid dehydrogenase. Therefore the stimulus to its induction is not represented by falling concentrations of circulating Δ^4 -3-oxo steroids.

These metabolic responses form a strikingly constant pattern, seen also after treatment with aminogluthethimide (Table 3) or compound CB-154 during pregnancy or after weaning of the litter during lactation (Rodway & Kuhn, 1975). We propose that they constitute a 'multiple-response pattern' which characterizes luteolysis and which may also include changes in glucose 6-phosphate dehydrogenase (EC 1.1.1.49), isocitrate dehydrogenase (EC 1.1.1.42) and 'malic' enzyme (EC 1.1.1.40) (Wiest *et al.*, 1968; Strauss & Stambaugh, 1974). By analogy with the pleiotypic growth response (Hershko *et al.*, 1971) these changes may be due to a single luteal mediator, as yet unidentified.

The remaining discussion assesses how far a surge of luteotrophin or the withdrawal of placental lactogen may be responsible for these luteal changes in normal late pregnancy, and how these may relate to prostaglandin.

Possible role of luteotrophin

Luteotrophin appears to affect 20α -hydroxy steroid dehydrogenase in two ways. On the one hand it induces the enzyme, both in pregnancy and in lactation. On the other hand, it partly inhibits the induction due to foetoplacental removal or to prostaglandin (Strauss & Stambaugh, 1974). This inhibitory property may also explain why the enzyme activity induced by gonadotrophin before day 17 of pregnancy was lower than that induced either by foetoplacental removal or even by gonadotrophin itself at late pregnancy. Thus the inhibitory response to gonadotrophin may disappear in late pregnancy. The activity of luteotrophin-releasing hormone in late pregnancy demonstrates the potential of an endogenous luteotrophin surge to induce the enzyme at this stage.

Nevertheless, the inability of hypophysectomy to delay the increase in 20α -hydroxy steroid dehydrogenase by more than 4 h at the end of normal pregnancy (Wiest *et al.*, 1968) casts doubt on any essential

participation by luteotrophin. Moreover the timing of the parturient luteotrophin surge is such that only minute increases of its plasma concentration appear to accompany, let alone precede, the increased activity of this enzyme (Rebar *et al.*, 1969; Midgley, 1973; Linkie & Niswender, 1972; Morishige *et al.*, 1973; Bast & Melampy, 1972; Labhsetwar & Watson, 1974). In summary, therefore, luteotrophin is unlikely to participate in the normal induction of 20α -hydroxy steroid dehydrogenase at late pregnancy.

Possible role of placental lactogen

Our experiments on the effects of foetoplacental removal and hysterectomy partly confirm and extend those of Wiest *et al.* (1968). They suggest that some inhibitory substance flows from the placenta to the ovaries, restraining the induction of 20α -hydroxy steroid dehydrogenase until nearly the end of pregnancy. The substance is carried humorally, rather than locally, in an amount proportional to the number of placentae. In contrast with Wiest *et al.* (1968), however, we were unable to replace this substance by prolactin, even at doses larger than those used by these authors. The possible interference by a luteolysin of uterine origin was unlikely since hysterectomy resulted in a similar time-course of enzyme induction that was also not inhibited by prolactin. Interference by the pituitary was also eliminated by the use of hypophysectomized rats. In the same way we were unable to block the enzyme induction that followed foetoplacental removal by injections of placental tissue under a wide variety of conditions. These attempts were extended to the mouse with similar results.

The inactivity of prolactin in these experiments is probably not due to its maladministration, since it was able to inhibit the induction of 20α -hydroxy steroid dehydrogenase after treatment of rats with compound CB-154 in early pregnancy (Rodway & Kuhn, 1975). It may be relevant that the experiments of Wiest *et al.* (1968) were carried out on rats that were several days earlier in pregnancy, since it has been recognized that the nature of the luteotrophic stimulus can vary according to the stage of pregnancy (Sairam & Li, 1973; Heap *et al.*, 1973). We suggest that the placental influence that normally restrains luteolysis at late pregnancy is not accounted for by placental lactogen, at least not on its own. Not to be discounted is the possibility of a placental destruction of a maternally derived luteolysin.

Possible role of prostaglandin

The participation of prostaglandin in the events leading to luteolysis is suggested by its ability, when injected, to induce 20α -hydroxy steroid dehydrogenase, and by the ability of indomethacin to block

the induction seen at normal parturition (Strauss & Stambaugh, 1974). However, the weak, or delayed, response to prostaglandin $F_{2\alpha}$ given before late pregnancy makes it unlikely that this hormone mediates the actions of gonadotrophin or foeto-placental removal. In some species uterine prostaglandin $F_{2\alpha}$ seems to be largely responsible for terminating luteal function (Denamur, 1974; Goding, 1974). In the case of the rat, however, such a hypothesis must be reconciled with the indirect evidence that favours a placental luteotrophin, as is described in the present paper.

Induction of 20 α -hydroxy steroid dehydrogenase by aminogluthethimide

Although aminogluthethimide might have other actions in the body, we assume that its effects described above are due to its interruption of steroid hormone synthesis. The precipitation of lactogenesis confirmed that the progesterone supply was indeed broken. Because oestrogen was the most effective substance in reversing the induction of 20 α -hydroxy steroid dehydrogenase by aminogluthethimide, it is likely that the induction was in fact due to a decreased concentration of this hormone, either in the circulation or locally in luteal or other tissue. Since administered progesterone only partially inhibited the enzyme induction, and only when given intraperitoneally, and could not be replaced by medroxyprogesterone, we suggest that it may act as a local oestrogen precursor.

Aminogluthethimide has therefore served to uncover an oestrogen-dependency during the last few days of pregnancy of the placental restraint on the induction of 20 α -hydroxy steroid dehydrogenase. Our studies have not supported the original hypothesis that the induction of 20 α -hydroxy steroid dehydrogenase is due to a decrease in circulating Δ^4 -3-oxo steroids.

The late appearance of oestrogen-dependence might indicate its participation in the normal mechanism for inducing this multiple response at the end of pregnancy. On the other hand, it could rather reflect the more general supportive role that oestrogen seems to play in all aspects of reproduction. Certainly there is no fall in circulating oestrogen concentrations at the end of pregnancy, but rather a large rise (Yoshinaga *et al.*, 1969; Shaikh, 1971; Labhsetwar & Watson, 1974). Although it appears that the oestrogen does not act via prolactin or the pituitary, nevertheless it might act via other tissues such as the fetuses or placentae; this might explain why aminogluthethimide did not induce 20 α -hydroxy steroid dehydrogenase in early pregnancy or during lactation. It is also possible that oestrogen acts directly to inhibit luteal prostaglandin synthesis. Thus aminogluthethimide was reported to accelerate the incor-

poration of arachidonic acid into prostaglandin by ovarian homogenates (Chasalow & Pharris, 1972), whereas prostaglandin $F_{2\alpha}$ is an efficient inducer of 20 α -hydroxy steroid dehydrogenase (Strauss & Stambaugh, 1974; Fuchs & Mok, 1974; the present paper, Fig. 7).

It is not yet clear what relationship the present findings may have to other luteotrophic actions of oestrogen that have been reported for the rat (Klein & Mayer, 1942; Hixon *et al.*, 1973; Takayama & Greenwald, 1973; Uchida *et al.*, 1973), some of which require the presence of the pituitary.

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