A Proposed Sequence of Hormones Controlling the Induction of Luteal 20 α -Hydroxy Steroid Dehydrogenase and Progesterone Withdrawal in the Late-Pregnant Rat

By DAVID HICKMAN-SMITH and NICHOLAS J. KUHN

Department of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

(Received 9 June 1976)

1. The previously reported induction of luteal 20 α -hydroxy steroid dehydrogenase by administration of aminoglutethimide to late-pregnant rats was shown to be unaffected by prior removal of the foetuses. Aminoglutethimide therefore does not act via the foetuses in this context. 2. The ability of injected oestrogen to prevent the above induction was lost by delaying the injection for 12 h after aminoglutethimide, although the increase in enzyme activity begins only after 24 h. 3. Induction of 20 α -hydroxy steroid dehydrogenase by foetoplacental removal on day 18 of pregnancy was inhibited by human choriongonadotropin, lutropin (luteinizing hormone) and pregnant-mare serum gonadotropin, but not by somatotropin (growth hormone), thyrotropin or follitropin (follicle-stimulating hormone). 4. Indomethacin blocked the normal induction of 20 α -hydroxy steroid dehydrogenase in late pregnancy and that caused by aminoglutethimide. It partially blocked that caused by human choriongonadotropin given on days 19-20 and that caused by 2-bromo- α -ergocryptine on days 5-6, but failed to block that caused by human choriongonadotropin on days 15-16 or by foetoplacental removal on day 18 of pregnancy. 5. These findings, and the control of progesterone synthesis in late pregnancy, are interpreted in terms of a sequence of hormonal or enzymic syntheses, each of which is inhibited by the product of the preceding synthesis.

Luteal 20 α -hydroxy steroid dehydrogenase is believed to play a critical role in luteolysis and progesterone withdrawal in the rat and mouse (Wiest *et al.*, 1968; Kuhn & Briley, 1970; Owen & Kuhn, 1971). The activity of this enzyme rises greatly at pro-oestrus, parturition and late lactation, but remains low at other stages of the reproductive cycle. During early pregnancy this low activity is apparently due to the influence of prolactin, since the enzyme can be induced by ergocornine or 2-bromo- α -ergocryptine, and this induction can be prevented by injected prolactin (Lamprecht *et al.*, 1969; Rodway & Kuhn, 1975b). At late pregnancy and during lactation, however, other factors, hitherto unidentified, appear to prevent induction of the enzyme (Rodway & Kuhn, 1975a). During late pregnancy the enzyme can be induced experimentally by administration of human choriongonadotrophin, sheep lutropin (luteinizing hormone), prostaglandin F_{2 α} or aminoglutethimide, or by removal of the conceptus. Induction after removal of the conceptus is partly prevented by the administration of choriongonadotropin, and that caused by aminoglutethimide is prevented by oestrogen and by indomethacin (an inhibitor of prostaglandin synthesis) (Rodway & Kuhn, 1975a).

Despite this plethora of conditions for the experimental induction of luteal 20 α -hydroxy steroid dehydrogenase, no acceptable explanation has so far been given for its natural induction at the end of pregnancy. The results in the present paper extend our understanding of the experimental inductions described above and, in particular, of conditions for their reversal. From these results, together with those in the papers cited above, a working hypothesis is proposed for the natural hormonal mechanism that restrains and finally initiates the induction of 20 α -hydroxy steroid dehydrogenase in the late-pregnant rat.

Materials and Methods

Young adult virgin rats from the Wistar-derived colony of this Department were caged singly with males and checked daily for the appearance of vaginal plugs. The morning on which a plug was first found was termed day 1 of pregnancy, and rats (at a weight of about 330 g) normally littered on days 22-23.

X-irradiation of temporarily exteriorized ovaries was performed on rats kept under Nembutal (4-5 mg/100 g body wt.) anaesthesia, otherwise protected by 5 mm lead shielding. Radiation was provided

by a Pantak constant-potential industrial radiography unit at the Birmingham Radiation Centre. For subsequent histological examination, ovaries were fixed in Bouin's fluid (Searle Scientific Services, High Wycombe, Bucks., U.K.), after which serial paraffin sections (7 μ m thick) were cut and stained with Haematoxylin and Eosin.

Where experiments were carried out on rats from which the foetuses had been surgically removed (Table 1), precautions were necessary to avoid actual or incipient dislodgement of the placentae, since this by itself is a signal for the induction of 20 α -hydroxy steroid dehydrogenase. In the procedure finally adopted, the foetuses were removed during the afternoon of day 18 of pregnancy through small uterine incisions (one per four foetuses, where possible) that were afterwards closed with silk thread. Drugs and hormones that were subsequently given on days 19 and 20 were preceded by subcutaneous injection of progesterone (6mg) 1–2h beforehand. Results from those few rats in which placental dislodgement nevertheless occurred were discarded.

20 α -Hydroxy steroid dehydrogenase was assayed as described by Kuhn & Briley (1970) at pH10, and activities were calculated as nmol of NADPH formed/min per ovary at this pH.

Extracts of placentae, liver and kidney were prepared by homogenization of each tissue in an equal volume of water and by the addition of NaOH to give pH approx. 9.5. After the homogenate had been stirred for 1 h at 0–4°C it was adjusted to pH7.4 and 0.9% NaCl by addition of HCl and NaCl, and was centrifuged for 30 min at 100000g_{av.}. The clear supernatant was used within 1 h for the ascorbic acid-depletion test (Parlow, 1961).

All other methods have been described previously (Rodway & Kuhn, 1975a).

Expression of results

Results are given as means $\pm$ S.E.M. with the numbers of rats in parentheses. The significance of differences between groups was assessed by Student's *t* test.

Materials

Pregnant-mare serum gonadotropin (Gestyl) and crude human choriongonadotropin (Pregnyl) were from Organon Laboratories Ltd., Morden, Surrey, U.K. Pure human choriongonadotropin (batch CR 119), sheep somatotropin (growth hormone) (NIH-GH-S11), sheep follitropin (follicle-stimulating hormone) (NIH-FSH-S11), sheep lutropin (NIH-LH-S18) and sheep thyrotropin (NIH-TSH-S8) were obtained by courtesy of the Center for Population Research of the National Institute of Child Health and Human Development, and of the

Pituitary Hormone Distribution Program, of the National Institutes of Health, Bethesda, MD, U.S.A. Indomethacin [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-ylacetic acid] was generously given by Merck, Sharp and Dohme Research Laboratories, Rahway, NJ, U.S.A. Aminoglutethimide (Elipten) was a kind gift from Dr. D. M. Burley, CIBA Research Laboratories, Horsham, Sussex, U.K. 2-Bromo- α -ergocryptine was kindly given by Professor E. Flückiger and Dr. H. Friedli, Pharmaceutical Division, Sandoz Ltd., Basel, Switzerland. Sources of other materials have been described previously (Rodway & Kuhn, 1975a).

Results

Induction of 20 α -hydroxy steroid dehydrogenase by aminoglutethimide

A single injection of aminoglutethimide on day 19 of pregnancy results in an activity of 20 α -hydroxy steroid dehydrogenase that begins to increase after 24h and reaches a high value at 48h. This is largely

Fig. 1. Effect of delayed oestrogen injection on the induction of luteal 20 α -hydroxy steroid dehydrogenase by aminoglutethimide

Rats received a single dose of aminoglutethimide (25 mg in 0.25 ml of 0.9% NaCl subcutaneously) on day 19 of pregnancy, and oestradiol-17 β (50 μ g in 0.2 ml of arachis oil, intraperitoneally) at various times thereafter. Progesterone (6 mg in 0.2 ml of arachis oil subcutaneously) was administered daily to suppress the abortifacient activity of oestradiol. 20 α -Hydroxy steroid dehydrogenase was assayed at 48 h. Results are means $\pm$ S.E.M. (indicated by vertical bars) with numbers of rats shown in parentheses.

Table 1. *Aminogluthethimide induction of 20 α -hydroxy steroid dehydrogenase in the absence of foetuses*

All rats were operated on day 18 of pregnancy to remove foetuses, and received progesterone (6 mg subcutaneously) daily, in addition to the treatments shown. Details are given in the Materials and Methods section. 20 α -Hydroxy steroid dehydrogenase was assayed on day 21 of pregnancy, 48 h after the administration of aminogluthethimide. The results of Student's *t* tests compared with the value for aminogluthethimide treatment alone are given as *P* values.

Treatment	Activity of 20 α -hydroxy steroid dehydrogenase (nmol/min per ovary)	<i>P</i>
None	7 $\pm$ 3 (5)	—
Aminogluthethimide (25 mg) at 0 h	238 $\pm$ 24 (11)	—
Aminogluthethimide (25 mg) at 0 h and oestrone (5 μ g) at 0 and 24 h	128 $\pm$ 46 (6)	0.05 > <i>P</i> > 0.01
Aminogluthethimide (25 mg) at 0 h and indomethacin (2 mg) at 0 and 24 h	39 $\pm$ 13 (7)	0.001 > <i>P</i>

Table 2. *Effect of indomethacin on the induction of luteal 20 α -hydroxy steroid dehydrogenase under different conditions*

A single daily dose of indomethacin (2 mg in 0.25 ml of 0.1 M-potassium phosphate buffer, pH 7.4) was given subcutaneously to each rat during the period of the experiment. This was the maximum tolerated dose. Normal late-pregnant rats were injected on days 19–22 inclusively and assayed early on day 23. Those rats that had already littered by this time were discarded. All other experimental inductions of 20 α -hydroxy steroid dehydrogenase were carried out as described by Rodway & Kuhn (1975a,b), and assays were performed 48 h after beginning the treatments. The result of a Student's *t* test is given as a *P* value for each indomethacin-treated group of rats versus its control group. Enzyme activities are also shown for untreated rats at the appropriate stage of pregnancy, so that the extent of induction by each treatment can be seen.

Condition of enzyme induction	Activity of 20 α -hydroxy steroid dehydrogenase (nmol/min per ovary)			<i>P</i>
	Without indomethacin	With indomethacin	Without enzyme induction	
Foetoplacental removal, day 18	293 $\pm$ 22 (8)	310 $\pm$ 33 (8)	*1.2 (5)	<i>P</i> > 0.5
Choriogonadotropin, days 15 and 16	100 $\pm$ 11 (6)	119 $\pm$ 9 (6)	12 $\pm$ 6 (6)	0.5 > <i>P</i> > 0.2
Choriogonadotropin, days 19 and 20	223 $\pm$ 17 (5)	164 $\pm$ 9 (6)	*1.2 (5)	0.02 > <i>P</i> > 0.01
2-Bromo- α -ergocryptine, days 5 and 6	218 $\pm$ 14 (8)	139 $\pm$ 16 (10)	58 $\pm$ 8 (6)	0.005 > <i>P</i> > 0.002
Normal late pregnancy	260 $\pm$ 7 (7)	72 $\pm$ 21 (9)	—	0.0005 > <i>P</i>
Aminogluthethimide, day 19	271 $\pm$ 21 (5)	41 $\pm$ 7 (5)	*1.2 (5)	0.0005 > <i>P</i>

* Activity in untreated rats on day 21 of pregnancy.

prevented by the simultaneous injection of oestrone, accompanied by progesterone to prevent its abortifacient activity (Rodway & Kuhn, 1975a). Fig. 1 shows the effect of progressively delaying the administration of oestrone. Up to 6 h after administration of aminogluthethimide, oestrone is still fully effective, but between 6 and 12 h it loses this ability. Thus by 12 h the corpus luteum has become committed to induction of the enzyme, although a further 12 h elapses before the induction actually begins. Clearly one or more processes must intervene between the point of action of aminogluthethimide and the appearance of 20 α -hydroxy steroid dehydrogenase.

Although the induction of the enzyme has completely escaped control by oestrogen 12 h after aminogluthethimide, it can still be inhibited by human choriogonadotropin. Thus, in a separate series of rats receiving aminogluthethimide on day 19 of pregnancy (0 h), the activity of 20 α -hydroxy steroid dehydrogenase at 48 h was 194 $\pm$ 13 nmol/min

per ovary (7) unless gonadotropin had been given (3 μ g each at 12, 24 and 36 h), when it was significantly (*P* < 0.0005%) decreased to 106 $\pm$ 12 nmol/min per ovary (8). This observation bears on the particular hormonal sequence proposed in Scheme 1 below.

Table 1 shows that 20 α -hydroxy steroid dehydrogenase was also induced by aminogluthethimide in rats from which the foetuses had been surgically removed, and that this induction was inhibited by oestrone and by indomethacin. Such inhibitions may be taken as evidence that the induction was not secondary to any incipient dislodgement of the placenta in these surgically treated animals, since induction of the enzyme by foetoplacental removal is not affected by either oestrone (Rodway & Kuhn, 1975a) or indomethacin (see below, and Table 2). It is clear therefore that none of the processes involved in the induction of 20 α -hydroxy steroid dehydrogenase by aminogluthethimide reside in the foetus, or are dependent on its presence.

It was not possible to induce 20α -hydroxy steroid dehydrogenase by other treatments that might be expected to prevent the synthesis or action of oestrogen. Injection of the anti-oestrogens CN-55945-27 [1-(2-{ p -[α -(p -methoxyphenyl)- β -nitrostyryl]phenoxy}ethyl)pyrrolidine monocitrate] (Parke-Davis Ltd.) (up to 22mg, intraperitoneally or subcutaneously) and testosterone (10mg, intraperitoneally or subcutaneously) on day 19 of pregnancy failed to induce any enzyme activity by day 21. In another series of rats the bilaterally exteriorized ovaries were exposed to X-irradiation at doses ranging from 3000 to 5000rd, at times ranging from day 6 to day 18 of pregnancy. In no case did this treatment itself induce 20α -hydroxy steroid dehydrogenase, nor did it prevent induction of the enzyme by injected choriogonadotropin. Histologically the ovaries showed extensive damage to the non-luteal elements, together with apparent complete integrity of the luteal cells. Although we cannot be sure that the action or formation of ovarian oestrogen was fully impaired by these treatments, the findings do not favour the participation of ovarian oestrogen synthesis in the control of luteal 20α -hydroxy steroid dehydrogenase.

Inhibition by indomethacin of induction of 20α -hydroxy steroid dehydrogenase by various treatments

The involvement of prostaglandin synthesis in physiological processes can be tested by examining the susceptibility of such processes to indomethacin, a potent inhibitor of prostaglandin synthesis. Strauss & Stambaugh (1974) have shown that indomethacin can block the normal induction of 20α -hydroxy steroid dehydrogenase which occurs at the end of pregnancy. The results in Table 2 confirm this, and show to what extent it can block the induction brought about by several other procedures. Induction by foetoplacental removal and by choriogonadotropin on days 15 and 16 of pregnancy is not affected

by the drug. The greater induction caused by choriogonadotropin given on days 19 and 20 of pregnancy (Rodway & Kuhn, 1975a) and that caused by 2-bromo- α -ergocryptine in early pregnancy are both partly blocked, suggesting that prostaglandins play a part in these inductions, but not an essential part. Finally, the induction caused by aminoglutethimide in late pregnancy is greatly inhibited by indomethacin, indicating an obligatory role of prostaglandin in this process.

Inhibition by polypeptide hormones of induction of 20α -hydroxy steroid dehydrogenase by foetoplacental removal

It was previously shown that hysterectomy or foetoplacental removal at late pregnancy induce maximal activity of luteal 20α -hydroxy steroid dehydrogenase within 2 days, and that crude preparations of human choriogonadotropin can partly prevent this induction, although prolactin cannot (Rodway & Kuhn, 1975a). The results in Table 3 extend this information, showing that sheep pituitary lutropin, pregnant-mare serum gonadotropin and highly purified human choriogonadotropin can all inhibit the induction of the enzyme, whereas somatotropin, follitropin and thyrotropin cannot. In the presence of increasing doses of lutropin or gonadotropin (Fig. 2) there was a progressively greater inhibition of enzyme induction, with an unexpectedly large inhibition at a particular low dose of each hormone. Thus 80% inhibition was given by 6 μ g (total dose) of gonadotropin. This complex pattern may reflect the paradoxical fact that these hormones can both promote and inhibit the induction of 20α -hydroxy steroid dehydrogenase. The greater potency of choriogonadotropin than that of lutropin may be ascribed to its longer half-life in rat plasma (Braunstein *et al.*, 1972; Ascoli *et al.*, 1975).

Preliminary experiments were made with the ovarian ascorbic acid-depletion test (Parlow, 1961)

Table 3. *Effect of polypeptide hormones on the induction of luteal 20α -hydroxy steroid dehydrogenase by foetoplacental removal*

Rats were operated on day 17 or 18 of pregnancy and 20α -hydroxy steroid dehydrogenase was assayed 48h later. During this period rats were either untreated or received the appropriate hormone in 0.9% NaCl, subcutaneously, with the total dose shown being divided into two or four equal doses. The result of a Student's *t* test is given as a *P* value for each experimental value versus its control value.

Activity of 20α -hydroxy steroid dehydrogenase (nmol/min per ovary)				
	Total dose	Without hormone	With hormone	<i>P</i>
Lutropin	0.6mg	299 $\pm$ 28 (6)	125 $\pm$ 4 (5)	0.0005 > <i>P</i> > 0.0002
Serum gonadotropin	75-150i.u.	251 $\pm$ 27 (8)	120 $\pm$ 14 (10)	0.0005 > <i>P</i> > 0.0002
Choriogonadotropin	0.6mg	274 $\pm$ 20 (9)	153 $\pm$ 19 (4)	0.005 > <i>P</i> > 0.002
Somatotropin	0.8mg	343 $\pm$ 20 (8)	294 $\pm$ 28 (7)	0.20 > <i>P</i> > 0.10
Follitropin	0.6mg	265 $\pm$ 23 (7)	265 $\pm$ 15 (7)	<i>P</i> > 0.50
Thyrotropin	0.4-0.6mg	239 $\pm$ 23 (6)	251 $\pm$ 14 (9)	<i>P</i> > 0.50

Fig. 2. Effect of different doses of lutropin (●) or choriogonadotropin (○) on the induction of luteal 20 α -hydroxy steroid dehydrogenase by foetoplacental removal

Experimental conditions were as in the legend to Table 3; 100% activity of 20 α -hydroxy steroid dehydrogenase was 253 ± 12 (23) and 268 ± 17 (21) nmol/min per ovary for choriogonadotropin and lutropin respectively. Results are means $\pm$ S.E.M. (shown by vertical bars) with the numbers of rats shown in parentheses.

to examine rat placentae directly for the presence of lutropin similar in properties to human choriogonadotropin (Table 4). Extracts of placentae from 18-day-pregnant rats significantly depleted the ovarian ascorbic acid content of recipient rats, whereas untreated serum or extracts of liver did not. A depletion was also obtained with extracts of kidney, possibly owing to the ability of this organ to take up and retain plasma lutropin (Braunstein *et al.*, 1972; Ascoli *et al.*, 1975).

Discussion

The induction of luteal 20 α -hydroxy steroid dehydrogenase at the end of pregnancy in the rat

was earlier proposed to be due to the removal of a restraint arising from the placenta (Rodway & Kuhn, 1975a). A mechanism predominantly of this sort, rather than one depending on the appearance of a luteolytic agent, had been foreshadowed by the observation that rats carrying two asynchronous litters underwent parturition only when the second litter reached term (Alloiteau *et al.*, 1963). Although the nature of this restraint was obscure, it did not seem to be a prolactin-like substance, as was originally considered by Wiest *et al.* (1968) and later by Kuhn (1971) and Sairam & Li (1973). With our present finding that induction of the enzyme by foetoplacental removal is specifically inhibited by

Table 4. Ovarian ascorbic acid content of rats injected with 0.9% NaCl (0.5 ml), serum, tissue extract or pure human choriogonadotropin

Values are given as percentages of saline-treated controls [100% is equivalent to 209 ± 6 (64) μg ascorbic acid per two ovaries] $\pm$ S.E.M., with numbers of rats in parentheses. The result of a Student's *t* test is given as a *P* value for each experimental value versus its control value.

Tissue	Stage of pregnancy (days)	Tissue equiv. injected (g or ml)	Ovarian ascorbic acid (%)		Significance (<i>P</i>)
			Control	Experimental	
Placenta	18	0.375	100 $\pm$ 5 (12)	84 $\pm$ 7 (12)	0.10 > <i>P</i> > 0.05
Placenta	18	0.75	100 $\pm$ 5 (12)	71 $\pm$ 5 (10)	0.001 > <i>P</i> > 0.0005
Kidney	15	0.62	100 $\pm$ 5 (12)	80 $\pm$ 7 (12)	0.05 > <i>P</i> > 0.02
Liver	18	0.48	100 $\pm$ 3 (16)	97 $\pm$ 6 (8)	<i>P</i> > 0.50
Liver	18	0.89	100 $\pm$ 3 (16)	96 $\pm$ 5 (14)	0.50 > <i>P</i> > 0.20
Serum	7	0.5	100 $\pm$ 11 (10)	96 $\pm$ 12 (10)	<i>P</i> > 0.50
Serum	18	1.0	100 $\pm$ 5 (11)	90 $\pm$ 7 (12)	0.50 > <i>P</i> > 0.20
Chorionic gonadotropin, 1 i.u. (0.086 μg)			100 $\pm$ 6 (4)	69 $\pm$ 8 (4)	0.02 > <i>P</i> > 0.01
Chorionic gonadotropin, 1 i.u. (0.86 μg)			100 $\pm$ 6 (4)	55 $\pm$ 2 (4)	0.0002 > <i>P</i> > 0.0001

choriogonadotropin, lutropin and pregnant-mare serum gonadotropin, hormones long known to have strongly overlapping properties, it seems likely that the putative placental factor is a lutropin of similar properties.

The fact that the hormones, when injected, did not fully inhibit the induction of 20 α -hydroxy steroid dehydrogenase might be construed as weakening the case for a placental lutropin, and certainly the experiments do not exclude the possible co-operation of a second hormone such as placental lactogen. We think it more likely, however, that periodic injections of heterologous hormones simply fail to simulate exactly the continuous release of endogenous placental hormone. The ability of placental extracts to deplete the ovarian content of ascorbic acid (Table 4) may be seen as preliminary, direct evidence for the presence of this lutropin.

Lutropin of pituitary origin may play a similar role earlier in pregnancy, since the administration of anti-lutropin antiserum at this stage markedly increases the activity of luteal 20 α -hydroxy steroid dehydrogenase (Loewit *et al.*, 1969) and the ratio of 20 α -hydroxypregn-4-en-3-one to progesterone in the ovarian venous blood (Moudgal *et al.*, 1972; Ford & Yoshinaga, 1975). These changes do not occur in later pregnancy. Although there is little doubt that prolactin or placental lactogen provides an additional restraint on the enzyme in early pregnancy, it remains unclear to what extent this may be true later on (Lamprecht *et al.*, 1969; Wiest *et al.*, 1968; Rodway & Kuhn, 1975a,b).

We interpret the induction of 20 α -hydroxy steroid dehydrogenase by aminoglutethimide as a response to a cessation of oestrogen synthesis in some compartment of the body, since it is effectively prevented by oestrogen itself. The response is not a direct one, since it is preceded by a 24 h lag (Rodway & Kuhn, 1975a) and escapes oestrogen control

within 12 h (Fig. 1). Because aminoglutethimide will not induce the enzyme in early pregnancy or in lactation, although other agents will, and because the induction is inhibited by administered gonadotropin, we consider it likely to act via a withdrawal of the placental lutropin proposed above. Moreover, since the induction by aminoglutethimide is prevented by indomethacin, whereas that caused by foetoplacental removal is not, we propose that synthesis of a prostaglandin links the two processes. Such a role of prostaglandin might account for the luteolytic action of injected prostaglandin F_{2 α} in the pregnant rat and for the slight delay in the subsequent induction of 20 α -hydroxy steroid dehydrogenase observed in early and middle pregnancy (Strauss & Stambaugh, 1974; Fuchs & Mok, 1974; Rodway & Kuhn, 1975a).

Scheme 1 illustrates the proposed relationships between these processes, and their bearing on progesterone withdrawal in the corpus luteum. It depicts a series of syntheses each of which is inhibited by the product of the preceding synthesis. The secretion of progesterone is inhibited by the action of 20 α -hydroxy steroid dehydrogenase, the induction of which is probably due to synthesis *de novo* (Wiest & Kidwell, 1969). It is proposed that induction of this enzyme is inhibited by a placental lutropin, synthesis of which is in turn inhibited by prostaglandin. During the last few days of pregnancy the synthesis of prostaglandin is, in this scheme, itself inhibited by oestrogen. It is envisaged that synthesis of the oestrogen may also occur within the placenta since surgical removal of the foetuses, another possible source, does not induce 20 α -hydroxy steroid dehydrogenase, yet allows induction by aminoglutethimide.

It is necessary to consider how far Scheme 1 accounts for other observations recorded in the literature and, insofar as it is correct, what change

Scheme 1. Linear sequence of hormonal and enzymic interactions, proposed to account for the maintenance and subsequent cessation of progesterone secretion in the late-pregnant rat

Arrows indicate possible sites of action of aminoglutethimide, indomethacin, foetoplacental removal, and lutropin, choriogonadotropin or pregnant-mare serum gonadotropin. 20α-OHSDH, 20α-hydroxy steroid dehydrogenase. Striped arrows denote inhibition.

may constitute the normal trigger for the cessation of placental lutropin synthesis in late pregnancy.

The ability of indomethacin to prevent the normal induction of 20α -hydroxy steroid dehydrogenase at parturition (Strauss & Stambaugh, 1974), together with its inhibition of progesterone secretion and the restoration of this by injected prostaglandin $F_{2\alpha}$ (Strauss *et al.*, 1975), certainly implicates prostaglandin synthesis as part of the normal mechanism. Nevertheless it is unclear to what extent this prostaglandin may be identified with the prostaglandin F measured in uterine-vein blood (Labhsetwar & Watson, 1974) or with the prostaglandin $F_{2\alpha}$ released by uteri taken from late-pregnant rats (Harney *et al.*, 1974), both of which increase at about the expected time. The growing realization that prostaglandins participate in many physiological processes makes it quite possible that separate functional pools operate in the placenta and uterus to control lutropin synthesis and myometrial contractility respectively. The idea that the luteolytic action of prostaglandin is due to inhibition of a lutropin was also proposed by Fuchs & Mok (1974), although they appeared to envisage an interaction with pituitary lutropin at the level of the corpus luteum. In support of either scheme is the fact that lutropin or choriogonadotropin can inhibit the induction of 20α -hydroxy steroid dehydrogenase by injected prostaglandin $F_{2\alpha}$, although these observations were not made in late pregnancy (Strauss & Stambaugh, 1974; Fuchs & Mok, 1974).

The delay of normal parturition that is caused by a suitably timed injection of oestrogen (Dukes *et al.*, 1974) can be accounted for by Scheme 1, and suggests that oestrogen withdrawal may also be a normal part of the luteolytic mechanism. In this case the maternal ovary may not be the site of oestrogen synthesis, since its production of oestrogen appears to increase rather than decrease at the end of pregnancy (Yoshinaga *et al.*, 1969; Shaikh, 1971; Labhsetwar & Watson, 1974). It is partly for this reason, and also because irradiation of the maternal ovary did not induce 20α -hydroxy steroid dehydrogenase, that we envisage the placenta as a more likely site of the critical oestrogen synthesis. It should be noted, however, that cessation of maternal oestrogen production for 12h, which would suffice to induce 20α -hydroxy steroid dehydrogenase (Fig. 1), could have been missed in the experiments cited above.

In conclusion, we suggest that the induction of luteal 20α -hydroxy steroid dehydrogenase is due to release from an hormonal restraint. The normal restraint may comprise pituitary prolactin in early pregnancy, pituitary lutropin (with or without placental lactogen) in mid-pregnancy, and predominantly a placental lutropin in late pregnancy. The formation and ultimate withdrawal of the latter hormone may be under the mutually inhibitory control of

prostaglandin and oestrogen within the placenta itself.

We gratefully acknowledge the advice of Dr. P. Fowles in the irradiation procedure, and the assistance of Mrs. Dorothy Clark and Miss Angela Drinkwater in the provision of pregnant rats. A part of the costs was defrayed by a grant from the Medical Research Council.

References

- Alloiteau, J. J., Psychoyos, A. & Acker, G. (1963) *C. R. Hebd. Séances Acad. Sci. Ser. D* **256**, 4284–4287
- Ascoli, M., Liddle, R. A. & Puett, D. (1975) *Mol. Cell. Endocrinol.* **3**, 21–36
- Braunstein, G. D., Vaitukaitis, J. L. & Ross, G. T. (1972) *Endocrinology* **91**, 1030–1036
- Dukes, M., Chester, R. & Atkinson, P. (1974) *J. Reprod. Fert.* **38**, 325–334
- Ford, J. J. & Yoshinaga, K. (1975) *Endocrinology* **96**, 329–334
- Fuchs, A. R. & Mok, E. (1974) *Biol. Reprod.* **10**, 24–38
- Harney, P. J., Sneddon, J. M. & Williams, K. I. (1974) *J. Endocrinol.* **60**, 343–351
- Kuhn, N. J. (1971) in *Lactation* (Falconer, I. R., ed.), pp. 161–176, Butterworths, London
- Kuhn, N. J. & Briley, M. S. (1970) *Biochem. J.* **117**, 193–201
- Labhsetwar, A. P. & Watson, D. J. (1974) *Biol. Reprod.* **10**, 103–110
- Lamprecht, S. A., Lindner, H. R. & Strauss, J. F. (1969) *Biochim. Biophys. Acta* **187**, 133–143
- Loewit, K., Badaway, S. & Lawrence, K. (1969) *Endocrinology* **84**, 244–251
- Moudgal, N. R., Behrman, H. R. & Greep, R. O. (1972) *J. Endocrinol.* **52**, 413–418
- Owen, A. L. & Kuhn, N. J. (1971) *J. Endocrinol.* **51**, 599–600
- Parlow, A. F. (1961) in *Human Pituitary Gonadotrophins* (Albert, A., ed.), pp. 300–312, Charles Thomas, Springfield, IL, U.S.A.
- Rodway, R. G. & Kuhn, N. J. (1975a) *Biochem. J.* **152**, 433–443
- Rodway, R. G. & Kuhn, N. J. (1975b) *Biochem. J.* **152**, 445–448
- Sairam, M. R. & Li, C. H. (1973) in *Hormonal Proteins and Peptides* (Li, C. H., ed.), vol. 1, pp. 102–169, Academic Press, London
- Shaikh, A. A. (1971) *Biol. Reprod.* **5**, 297–307
- Strauss, J. F. & Stambaugh, R. L. (1974) *Prostaglandins* **5**, 73–84
- Strauss, J. F., Sokoloski, J., Caploe, P., Duffy, P., Mintz, G. & Stambaugh, R. L. (1975) *Endocrinology* **96**, 1040–1043
- Wiest, W. G. & Kidwell, W. R. (1969) in *Gonads* (McKerns, R., ed.), pp. 295–325, Appleton-Century-Crofts, New York
- Wiest, W. G., Kidwell, W. R. & Balogh, K. (1968) *Endocrinology* **82**, 844–859
- Yoshinaga, K., Hawkins, R. A. & Stocker, J. F. (1969) *Endocrinology* **85**, 103–112