Immunoreactive cytochrome P-450_{17α} in rat and guinea-pig gonads, adrenal glands and brain

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Summary. The cytochrome P-450_{17α}-hydroxylase, 17→20 lyase (P-450_{17α}) is the key enzyme responsible for the biosynthesis of androgens in steroidogenic organs. Its cellular localization has been examined with an immunohistochemical technique.

In immature rat ovary, P-450_{17α} was first detected in sparse interstitial cells on postnatal Day 8. The number of immunoreactive interstitial cells increased thereafter and the intensity of P-450_{17α} staining in these cells was highest at 3 weeks of age. The intensity of staining then started to decline and was very faint at Day 35. From 6 weeks on, the distribution of immunoreactive P-450_{17α} was of the adult type: it was detected exclusively in the thecal cells of the large antral, preovulatory, follicles. P-450_{17α} was not detectable during pregnancy except on the day of parturition, when thecal cells were transiently immunoreactive. The staining had vanished 24 h after delivery.

Human chorionic gonadotrophin (hCG), injected into immature females on Days 24 to 26, induced P-450_{17α} prematurely in thecal cells. When injected on Days 12 to 14 of pregnancy, hCG also induced P-450_{17α} in the thecal cells surrounding the largest follicles, whereas the interstitial and luteal cells were not immunostained. The anti-progestin RU486, injected on Day 16 of pregnancy, reinstated P-450_{17α} (and P-450_{sec}) immunoreactivity in the thecal cells. Oestradiol selectively suppressed P-450_{17α} expression in the thecal cells of RU486-treated females.

In immature guinea-pig ovary, P-450_{17α} was immunostained in thecal cells, not in interstitial cells, although the interstitial cells expressed the Δ5-3β-hydroxysteroid dehydrogenase.

P-450_{17α} was also immunolocalized in the Leydig cells of rat and guinea-pig testes, and in the guinea-pig adrenal cortex (zonae fasciculata and reticularis), but not in the rat adrenal cortex. P-450_{17α} was not detectable in the brain of either rat or guinea-pig.

Keywords: cytochrome P-450_{17α}; cytochrome P-450_{sec}; Δ5-3β-hydroxysteroid dehydrogenase; immunohistochemistry; ovary; testis; adrenal cortex; brain; rat; guinea-pig

Introduction

Pregnenolone (P) and dehydroepiandrosterone (D) are 3β-hydroxy-Δ5-steroids, derived from cholesterol by side-chain cleavage, and are precursors of steroid hormones secreted by steroidogenic glandular cells. These 3β-hydroxy-Δ5-steroids accumulate in brain through mechanisms at least partly independent of peripheral sources (review in Robel *et al.*, 1987). The rat brain contains the cholesterol side-chain cleavage system. Immunohistochemical studies have been performed with specific antibodies to bovine adrenal cytochrome P-450_{sec} and the enzyme was localized in the white matter throughout the brain (Le Goascogne *et al.*, 1987). This observation led to the biochemical

demonstration of P-450_{sc} activity in oligodendrocyte mitochondria (Hu *et al.*, 1987) and glial cell cultures (Jung-Testas *et al.*, 1989).

Both pregnenolone and progesterone may be 17 α -hydroxylated and may then undergo scission of the C-17,20 carbon bond to yield D and androstenedione, respectively. It is now well demonstrated that these reactions are mediated by a single enzyme, P-450_{17 α} (Miller, 1988; Namiki *et al.*, 1988).

Antibodies to P-450_{17 α} have been produced in rabbits immunized with the enzyme purified from pig testis (Rodgers *et al.*, 1986a; Sasano *et al.*, 1989a) and from guinea-pig adrenal cortex (Shinzawa *et al.*, 1988).

In the present study, we investigated by immunohistochemistry the expression and localization of P-450_{17 α} in rat and guinea-pig ovary, testis, adrenal gland and brain. In rat ovary, several physiological stages were studied: ontogenesis, oestrous cycle, pregnancy and parturition. In immature and pregnant females, human chorionic gonadotrophin (hCG) was injected as a substitute for luteinizing hormone (LH), which selectively stimulates androgen biosynthesis and P-450_{17 α} activity in rat ovary (Khan *et al.*, 1987).

RU486 is a progesterone antagonist (Baulieu & Segal, 1985). It brings about preterm birth in rats (Garfield *et al.*, 1987) and thus allows comparison of the effects of abortion and delivery on ovarian steroidogenic enzymes (Le Goascogne *et al.*, 1989a).

Materials and Methods

Animals

83 Sprague-Dawley rats and 6 Hartley guinea-pigs, males and females, were obtained from Iffa-Credo (L'Arbresle, France). Rats were 1 day to 17 weeks old, including pregnant and post-partum females. Guinea-pigs were 15 days old (Table 1). The stage of the oestrous cycle was determined by vaginal smears in adult female rats.

Hormonal treatments

hCG. Eight immature female rats received subcutaneous injections of 0.5 iu hCG (Organon) in the morning and evening of Days 24 and 25 and in the morning of Day 26; 5 control females received the phosphate-buffered saline (PBS) vehicle. They were killed on the afternoon of Day 26; hCG (1.5 iu) was also injected into 5 pregnant rats at 10:00 h and 16:00 h on Days 12 and 13 of pregnancy and at 10:00 h on Day 14. They were killed 2 h after the last injection. The injected doses of hCG were as described by Khan *et al.* (1987).

Progesterone. Three immature female rats received subcutaneous injections of progesterone (1.5 mg in 0.25 ml sesame oil) on Days 19, 20 and 21 and were killed on Day 22.

RU486. Five pregnant rats were given the progesterone antagonist RU486 (10 mg/kg) on Day 16 of pregnancy. Three of them received, in addition, oestradiol benzoate (100 μ g daily) on Days 16 and 17; RU486 and oestradiol benzoate (kindly provided by Roussel-Uclaf, Romainville, France) were injected under the skin in sesame oil solution (Le Goascogne *et al.*, 1989a).

Preparation of tissues

Ovaries, testes, adrenal glands and brains were taken from animals killed by decapitation under phenobarbital anaesthesia. Several fixation procedures were compared including intracardiac perfusion with periodate lysine paraformaldehyde (PLP) and fixation by immersion for 24 h at room temperature in PLP, Carnoy's fluid (absolute ethanol/chloroform/glacial acetic acid, 6:3:1 by vol.) or methanol and glacial acetic acid (3:1, v/v). None of these methods allowed the immunohistochemical detection of P-450_{17 α} in the brain, but they were all suitable for the steroidogenic glands. Perfusion could be omitted and the best results were obtained by fixation in methanol and glacial acetic acid.

After fixation, tissues were embedded in paraffin and sections 7 μ m thick were mounted on albumin-coated glass slides. For brain, 3000 coronal 7- μ m sections were cut and every 50th section was immunostained.

Immunochemical reagents

P-450_{17 α} was purified from guinea-pig adrenal microsomes (Kominami *et al.*, 1982). The resulting preparation was homogeneous in sodium dodecyl sulphate polyacrylamide gel electrophoresis (Shinzawa *et al.*, 1988). The

Table 1. Age, number of animals and organs studied in rats and guinea-pigs treated with hormones

		Animals		Number of			
		♀	♂	Ovaries (treatment)	Testes	Adrenals	Brain
Rats							
Immature	Age (days)						
	1	2	2	4	4	8	
	8	2		4			
	15	2		4			
	20		2		4		
	21	2		4			
	22	1		2 (control)			
		3		6 (progesterone)			
	26	5		10 (control)			
		8		16 (hCG†)			
	30	3		6			
	31		1			2	
	35	2		4			
Adult	Age (weeks)						
	6	10		20			2
	11	2	2	4	4	2	
	17	2	2	4			2
Pregnant	Gestational age (days)*						
	3	1		2			
	4	4		8			
	6	1		2			
	7	1		2			1
	9	2		4			
	14	2		4 (control)			
		5		10 (hCG†)			
	17	3		6			
	18	2		4 (control)			
		2		4 (RU486)			
		3		6 (RU486 + oestradiol benzoate)			
	21	1		2			
	22	2		4			
	1 day post partum		1		2		
Guinea-pigs (15 days old)		2	4	4	6	2	2

*Day of insemination designated as Day 1 of pregnancy.

†hCG human chorionic gonadotrophin.

immunoglobulin G (IgG) against the purified cytochrome was purified from the antisera of male white rabbits by chromatography on DEAE cellulose and Sephadex G200. The IgGs were reactive only to P-450_{17α} in guinea-pig adrenal microsomes (P-450_{C21} was not labelled). The specificity of the IgGs was further shown by the inhibitory effect on the 17α-hydroxylase reaction of guinea-pig adrenal microsomes. Rabbit antibodies to bovine cytochrome P-450_{9c} were prepared as described by Le Goascogne *et al.* (1987). Rabbit IgGs against human placental microsomal 3β-hydroxysteroid dehydrogenase-isomerase (3β-HSD) were kindly provided by I. Mason, South Western Medical Center, Dallas. The purified enzyme was provided by Drs Strickler and Thomas of Washington University of St Louis. Biotinylated goat anti-rabbit immunoglobulin G and avidin-biotin-peroxidase complex (Vectastain Elite reagents) were obtained from Vector Laboratories, Burlingame, CA, USA.

Immunohistochemical and histological staining

Deparaffinized and rehydrated sections were rinsed in PBS and incubated in 3% goat nonimmune serum for 20 min. After rinsing in PBS, the anti-P-450_{17α} IgGs were used at 10–60 µg/ml at room temperature for 2 h. The biotinylated goat anti-rabbit IgG secondary antibody was then applied at a 1:200 dilution for 30 min, followed by the avidin-biotin-peroxidase complex, at a dilution of 1:100, for 30 min. The peroxidase activity was revealed by 3,3'-diamino-benzidine tetrahydrochloride (0.5 mg/ml) in the presence of H₂O₂ (0.01% in Tris buffer, pH 7.4).

Sections were not counterstained. They were rinsed, dehydrated and mounted. Controls were run on adjacent sections placed on the same histological slide and included IgGs from nonimmune serum, dilution of specific antibodies down to extinction of staining and presaturation of specific antibodies with purified guinea-pig P-450_{17 α} (24 h at 4°C) (Figs 1, 3b and d and 7b). IgGs against P-450_{sc} were diluted at 20 μ g/ml in PBS, and IgGs against 3 β -HSD were diluted at 4 μ g/ml. Azan's staining was performed for histological characterization of tissues.

Fig. 1. Specificity of cytochrome P-450_{17 α} immunostaining in a 21-day-old rat ovary, (a), (b) and (c) and 15-day-old guinea-pig ovary, (d), (e) and (f). In (a) and (d), the anti-P-450_{17 α} IgGs were used at 20 μ g/ml. Biotinylated goat anti-rabbit secondary antibody was applied at 1:200 dilution. After addition of avidin–biotin–peroxidase, the peroxidase activity was revealed with diaminobenzidine. In (b) and (e), the anti-P-450_{17 α} IgGs were preincubated with purified cytochrome P-450_{17 α} . In (c) and (f), nonimmune IgGs were used instead of specific IgGs. (a), (b) and (c) $\times$ 60; (d), (e) and (f) $\times$ 75.

Results

Ovary of immature rats

No immunoperoxidase reaction was observed from birth until postnatal Day 8, when sparse secondary interstitial cells were stained (not shown). Many more immunoreactive interstitial cells appeared at Day 15 (Fig. 2a), their number and intensity of staining reached a maximum by Days

21–22. At this stage, they predominated around the secondary follicles in the central region of the ovary (Fig. 2b and e) (12 ovaries), whereas few scattered thecal cells surrounding the largest tertiary, antral follicles were only slightly immunostained (Fig. 2d). Thereafter, the intensity of immunostaining decreased steadily; at Day 26 the interstitial cells still labelled by the immunoperoxidase reaction were located around atretic follicles (Fig. 2c and f), whereas the staining had almost completely disappeared by Days 30–35 (not shown) (10 ovaries).

Fig. 2. Ontogenesis of cytochrome P-450_{17α} in a rat ovary: (a) Day 15; many interstitial cells are immunoreactive, granulosa cells and oocytes are not stained; (b) Day 21; intensity of staining of the interstitial cells is highest, (c) Day 26; immunoreactivity of interstitial cells is less than on Day 21; follicles surrounded by the interstitial cells are undergoing atresia; (d) 21-day-old rat; interstitial cells surrounding secondary follicles are heavily immunostained, while only some thecal cells around the large antral follicles at ovarian periphery are faintly stained. All sections were treated in identical conditions, to allow reliable comparison of staining intensities (dilution of IgGs 1:3000); (e) histological staining (Azan) on Day 21 showing healthy follicles; (f) histological staining on Day 26 showing atretic follicles; (a), (b), (c), (e) and (f) $\times 100$; (d) $\times 40$.

Ovary of cyclic rats

After puberty, the staining of the interstitial cells had completely disappeared. Immunoreactive P-450_{17α} was only detected in the thecal cells surrounding the large Graafian preovulatory follicles

(Fig. 3a). The staining was heterogeneous, varying from nil to very strong (28 ovaries). Neither the thecal cells surrounding secondary and small antral follicles nor the oocytes, granulosa cells and luteal cells were immunostained.

Fig. 3. Immunostaining of cytochrome P-450_{17α} in adult rat ovaries: (a) 6 weeks-old at pro-oestrus; thecal cells surrounding the Graafian follicles are immunostained, but interstitial cells are not; (b) adjacent section treated with nonimmune IgGs; (c) Day 22 of pregnancy; thecal cells, unstained during the entire course of pregnancy, are now immunoreactive; (d) adjacent section treated with nonimmune IgGs. All $\times 80$.

Ovary of pregnant rats

None of the rat ovarian cell types was immunostained in rat ovary (34 ovaries) during the entire course of pregnancy, but transient and intense staining occurred on Day 22, the last day of pregnancy, in the thecal cells pertaining to Graafian follicles and in the hypertrophied thecal cells surrounding atretic follicles (Fig. 3c) (4 ovaries), then vanished within 24 h following parturition.

Hormonal treatments

hCG. Injection of hCG into immature female rats on Days 24, 25 and 26 produced premature and intense immunostaining of most thecal cells surrounding the antral follicles, either small or large (Fig. 4a) (16 ovaries). The staining of interstitial cells was unaffected by hCG.

After injection of hCG to female rats on Days 12, 13 and 14 of pregnancy, 10 ovaries were removed. The thecal cells surrounding the large antral follicles were selectively immunostained with anti-P-450_{17α} IgGs (Fig. 4b), although in a heterogeneous manner (Fig. 4c and d). Again, neither the corpora lutea nor the granulosa cells, nor the interstitial cells were immunoreactive. Tissues of the control, untreated animals were not immunostained (Fig. 4e).

RU486 and oestradiol. Treatment with the progesterone antagonist RU486 for 48 h, beginning on Day 16 of pregnancy, resulted in the reappearance of the immunohistochemical staining of P-450_{sec}, thus confirming previous observations (Le Goascogne *et al.*, 1989a), and of P-450_{17α} in the thecal cells (Fig. 5a and b). In pregnant rats injected with RU486 and oestradiol, the immunostaining of P-450_{sec} was at least maintained (Fig. 5c), whereas that of P-450_{17α} was completely abolished (Fig. 5d) (6 ovaries).

Progesterone. Treatment of immature rats on Days 19–21 with progesterone was ineffective in suppressing the strong immunohistochemical staining of the interstitial cells with anti-P-450_{17α} IgGs (6 ovaries).

Immature guinea-pig ovary

In the ovaries taken from 15-day-old guinea-pigs, the thecal cells surrounding the follicles, whatever their stage of development, were immunostained with anti-P-450_{17α} IgGs (Fig. 6a). The staining tended to be stronger and more diffuse in large antral follicles than in small ones, but it was definitely heterogeneous (Fig. 6b). The interstitial cells were not immunostained with anti-P-450_{17α} IgGs, but they strongly reacted with IgGs to 3β-HSD (Fig. 6c); even the staining with anti-3β HSD IgGs was stronger in interstitial than in thecal cells. Neither the granulosa cells nor the oocytes were stained with anti-P-450_{17α} IgGs.

Rat and guinea-pig testis

The Leydig cells were intensely immunostained with anti-P-450_{17α} IgGs in testis of newborn rats (Fig. 7a), a period of transient testosterone secretion. However, the immunostaining was not lower in 3-week-old rats and was not higher in adults (not shown). The Leydig cells were also intensely stained in 15-day-old guinea-pigs (Fig. 7c). In contrast to the thecal cells in the ovary, the staining of the Leydig cells was not heterogeneous. None of the cell types, including Sertoli cells, was immunostained in the seminiferous tubules.

Rat and guinea-pig adrenal glands

In guinea-pig, the cells of the adrenal cortex were immunostained with anti-P-450_{17α} IgGs confirming previous observations (Shinzawa *et al.*, 1988). The staining displayed a zonal distribution; the zona glomerulosa cells appeared completely devoid of antigen (while they were positive for P-450_{sec} and 3β-HSD), in contrast to the zona fasciculata and reticularis, where the intensity of

Fig. 4. Immunostaining of cytochrome P-450_{17 α} in ovaries of rats treated with human chorionic gonadotrophin (hCG): (a) immature rats; 0.5 iu hCG was injected twice a day on Days 24 and 25 and on the morning of Day 26; ovaries were taken in the afternoon of Day 26. The thecal cells are selectively immunostained. (b) pregnant rats; 1.5 iu hCG was injected twice a day on Days 12 and 13 of pregnancy and on the morning of Day 14; ovaries were taken 2 h after the last injection. Contrary to the secondary and small antral follicles, the large antral follicles are surrounded by intensely stained thecal cells. The interstitial cells (I), the luteal cells in corpora lutea (L) and the granulosa cells (G) remain unstained. (c), (d) higher magnification, showing the heterogeneous staining of thecal cells' cytoplasm. (e) ovary taken from a control rat (Day 14 of pregnancy) not injected with hCG. (a), (b) and (e) $\times 60$; (c) and (d) $\times 600$.

Fig. 5. Effects of RU486 and oestradiol benzoate on P-450_{scc} and P-450_{17α} on ovaries of pregnant rats. (a) and (b) 10 mg RU486/kg was injected on Day 16 of pregnancy and ovaries were taken 48 h later. (c) and (d) animals were treated with RU486 as in (a) and (b) together with 100 µg of oestradiol benzoate on Days 16 and 17. (a) and (c) P-450_{scc}; (b) and (d) P-450_{17α} immunostaining. RU486 induces immunoreactive P-450_{scc} in thecal and interstitial cells (a) and P-450_{17α} in thecal cells (b). Oestradiol benzoate inhibits the effect of RU486 on P-450_{17α} (d) but not on P-450_{scc} (c). All × 60.

Fig. 6. Immunostaining in 15-day-old guinea-pig ovary. (a) and (b) cytochrome P-450_{17α}. (a) thecal cells are intensely stained, while all other cell components including interstitial tissue are negative. (b) higher magnification shows that thecal cells are irregularly stained and that interstitial tissue (I) is not. (c) 3β-hydroxysteroid dehydrogenase; interstitial cells are immuno-reactive, contrary to thecal (T) and to granulosa cells (G). (a) × 50; (b) and (c) × 500.

Fig. 7. Immunostaining of cytochrome P-450_{17α} in testis of (a) 1-day-old rat; the cytoplasm of Leydig cells exhibits an intense immunostaining, but their nuclei, the other stromal cells and the seminiferous tubular cells do not; (b) control section of 1-day-old rat incubated with non-immune IgGs; (c) 15-day-old guinea-pig. The localization of staining is like that in the rats. (a) and (b) × 300; (c) × 120.

staining was strongest in the outer layer of the zona fasciculata and gradually decreased along the inner layer of the zona fasciculata and the zona reticularis; the chromaffin cells in the adrenal medulla were not immunostained, but were separated by thin tracts of adrenocortical cells strongly immunostained with anti-P-450_{17α} IgGs as well as by anti-P-450_{sc} and 3β-HSD IgGs (not shown).

In rats, none of the zones of the adrenal cortex reacted with anti-P-450_{17α} IgGs, but they were all stained with anti-P-450_{sc} and anti-3β-HSD (Dupont *et al.*, 1990b) IgGs, including the adrenocortical cells interspersed in the medulla (Fig. 8).

Fig. 8. Immunostaining in adrenal gland of 11-week-old male rat. Cytochromes (a) P-450_{sc} and (b) P-450_{17α}; (c) 3β-hydroxysteroid dehydrogenase. G, zona glomerulosa; F, zona fasciculata; R, zona reticularis; M, medulla. All $\times 45$.

Rat and guinea-pig brain

Serial sections of the entire brain were examined every 350 μ m. They were devoid of cells and structures immunostained with anti-P-450_{17α} IgGs, even with concentrations of antibodies 3 times larger than those used for the steroidogenic glands, whatever the sex, stage of development or ovarian activity.

Discussion

The present work indicates that antibodies produced against P-450_{17α} from guinea-pig can be used for the immunohistochemical localization of the rat enzyme, indicating that the enzymes of both species share common epitopes, due to the high sequence similarities between rat and other mammalian P-450_{17α} enzymes (Namiki *et al.*, 1988).

Therefore, the failure to demonstrate the presence of the P-450_{17α} antigen in rat brain does not seem to be related to the heterologous character of the immunocytochemical reaction. It is in accordance with the lack of conversion of pregnenolone to dehydroepiandrosterone by incubation of rat brain preparations and by cultures of glial cells (Robel *et al.*, 1991). The immunocytochemical reaction for P-450_{sc} was also heterologous, but gave positive results (Le Goascogne *et al.*, 1987) that were ultimately confirmed by biochemical approaches (Hu *et al.*, 1987; Jung-Testas *et al.*, 1989). The search for P-450_{17α} antigen in the guinea-pig brain with homologous antibody was also unsuccessful.

Our data confirm a previous report concerning the localization of P-450_{17 α} in guinea-pig adrenal cortex (Shinzawa *et al.*, 1988). A similar zonal distribution occurs in pig adrenal glands (Sasano *et al.*, 1989a). The enzyme was not detected by us in rat adrenal cortex or in mouse adrenal gland (Perkins & Payne, 1988), because adrenal glands of rodents produce little, if any, 17 α -hydroxysteroids or androgens. However, a slight, but definite, P-450_{17 α} activity has been documented in rat adrenal glands (Johnson, 1979). The amount of enzyme is too small to be detected by an immunohistochemical approach.

The developmental expression of P-450_{17 α} has been investigated previously. The enzyme is present in fetal testis at all gestational ages (Waterman & Simpson, 1989) and has been purified from testis of newborn pigs (Nakajin & Hall, 1981). We have observed that the Leydig cells were selectively immunostained by antibodies to P-450_{17 α} in the testis, not only of newborn rats, which are known to secrete testosterone transiently (Corbier *et al.*, 1978), but also of immature rats and guinea-pigs. Post-pubertal Leydig cells are immunostained with antibodies against P-450_{17 α} in rats (present work) and pig testis (Sasano *et al.*, 1989a) and by antibodies to P-450_{sc} in human (Le Goascogne *et al.*, 1989b) and rat testis (C. Le Goascogne & M. Gouézou, unpublished results).

Little is known about the expression of cytochrome P-450_{17 α} protein in the ovary throughout development. A definite pattern of P-450_{17 α} expression occurs in the ovary of immature rats. The number and intensity of immunoreactive secondary interstitial cells exhibit a sharp maximum at 3 weeks of age. Serum LH is slightly increased during the 2nd and 3rd weeks of life, culminating at Day 21 (Döhler & Wuttke, 1975) and falling to low concentrations thereafter until puberty. Therefore, LH might be responsible for the induction of P-450_{17 α} , including the slight staining of cells surrounding large antral follicles (Bogovich & Richards, 1982).

The P-450_{sc} antigen has the same cellular localization and time course of appearance as P-450_{17 α} , but, once induced, extent and intensity of immunostaining P-450_{sc} remains about the same (Le Goascogne *et al.*, 1989a).

Immunoreactive 3 β -HSD was also detected in the cells labelled with the antibodies to P-450_{sc} and to P-450_{17 α} . In addition, after the 3rd postnatal week, granulosa cells of the rat large antral follicles expressed the 3 β -HSD antigen. In the ovary of immature guinea-pigs, 3 β -HSD was more intensely immunostained in interstitial than in thecal cells, and not at all in granulosa cells (present work), whereas thecal and some granulosa cells are immunoreactive in adult ovarian follicles (Dupont *et al.*, 1990a). Therefore, in rats, the same cell type contains the enzymes responsible for androstenedione synthesis (interstitial cells of immature females, thecal cells in Graafian follicles of mature females), whereas the thecal cells of the guinea-pig ovary may need the co-operation of interstitial cells to convert Δ 5-3 β -hydroxysteroids (namely dehydroepiandrosterone) to Δ 4-3-ketosteroids (androstenedione).

In cyclic female rats, P-450_{17 α} was detected immunohistochemically in the thecal cells of pre-ovulatory follicles. We previously reported that P-450_{sc} antigen was expressed in the interstitial cells, the thecal cells lining the preovulatory follicles and in the luteal cells of cyclic females (Le Goascogne *et al.*, 1989a). Recent studies have shown that the increase in follicular P-450_{17 α} activity which occurs at pro-oestrus is associated with increased content of the enzyme, as measured by Western blots using a P-450_{17 α} antibody, and increased content of P-450_{17 α} mRNA, as measured by filter hybridization using a P-450_{17 α} cDNA (Richards & Hedin, 1988).

There is general agreement that the rise of serum LH is responsible for the increased P-450_{sc} and P-450_{17 α} concentrations in thecal cells at pro-oestrus, (Erickson *et al.*, 1985). The P-450_{sc} gene stays turned on after ovulation, in luteinized granulosa and thecal cells, and appears to be constitutively expressed in rat corpora lutea, in a cAMP-independent manner (Goldring *et al.*, 1987). In contrast, P-450_{17 α} gene expression is abruptly turned off after the LH surge and subsequent ovulation. One interpretation of these results, confirmed by our immunohistochemical observations, is that low doses of LH or hCG increase P-450_{17 α} concentration, whereas the LH surge or ovulatory doses of hCG reduce the content of the enzyme and the transcription of the P-450_{17 α} gene (Hedin *et al.*, 1987). Another interpretation of the results on P-450_{sc} and P-450_{17 α} gene expression resides

in the intervention of inhibitory factors, which include prolactin, epidermal growth factor, gonadotrophin-releasing hormone and oestradiol. However, the two last factors are the only ones known to inhibit LH-stimulated androgen synthesis by ovarian cells selectively at the level of P-450_{17α} enzyme activity (Magoffin & Erickson, 1982; Erickson *et al.*, 1985). Our results on the effects of oestradiol on P-450_{17α} antigen expression in the ovaries of pregnant rats treated with RU486 favour the inhibitory role of oestradiol on P-450_{17α} gene expression, as already indicated for the adrenal enzyme (Johnson, 1979) and documented for the testicular enzyme (Nozu *et al.*, 1981).

The production of testosterone and oestradiol by the rat ovary, including the corpus luteum of pregnancy, is definite, but very small. Injection of hCG on Days 12–14 results in a dramatic increase in the secretion of androgen and oestrogen (Khan *et al.*, 1987). The stimulation of P-450_{17α} activity occurs several hours after the injection of hCG, suggesting an effect on enzyme synthesis. Indeed, our immunohistochemical data indicate that, 2 days after hCG injection, a large accumulation of P-450_{17α} antigen occurs in the thecal cells of large antral follicles. As the corpus luteum of rats derives from both granulosa and theca interna cells, we anticipated that immunocytochemistry might reveal some P-450_{17α} + cells after hCG stimulation. This was not the case since, despite the use of the highly sensitive avidin–biotin–peroxidase Elite reagent, P-450_{17α} was not detected in the corpus luteum, although the latter has a definite androgen production; neither was P-450_{17α} antigen detected in the cow corpus luteum by an immunofluorescence procedure (Rodgers *et al.*, 1986b), but was immunostained in the luteinized theca cells in human corpus luteum (Sasano *et al.*, 1989b).

The reappearance of P-450_{17α} antigen immediately before parturition is in accordance with the resumption of follicular development which occurs at this stage (Yoshinaga *et al.*, 1969). A premature reappearance was triggered not only by hCG injections, but also by treatment with RU486 for 48 h. This effect of RU486 was previously shown to occur for P-450_{ssc} expression and was interpreted as the relief of the down-regulatory role of progesterone (Le Goascogne *et al.*, 1989a). This conclusion was supported by the progressive disappearance of P-450_{ssc} immunoreactivity in the ovaries of pseudopregnant rats and of cyclic female rats after 9 days of progesterone treatment. The lack of effect of progesterone on P-450_{17α} immunostaining of the interstitial cells from immature rat ovaries might be due to the short duration of treatment.

In conclusion, P-450_{17α} is transiently expressed in ovarian interstitial cells during the development of immature females and in the thecal cells during the last stages of follicular maturation. This phenomenon might be related to the sequential roles of the inducer (LH-hCG), and of inhibitors, among which oestradiol is a good candidate.

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