The role of hormone receptors in development and puberty

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The development of radionuclide labelling of hormones has greatly facilitated the study of their interactions with their receptor sites in target tissues. Although in virtually all cases the real nature of the receptor site is unknown, nevertheless it is possible to study hormone–receptor interactions qualitatively and quantitatively. This paper is devoted to some recent studies of steroid– and gonadotrophin–receptor reactions in central and peripheral tissues during development, mainly in rodents. The available evidence has been organized to examine what part these receptors may play in the attainment of sexual maturity.

Steroid hormone receptors

The evidence for steroid hormone receptors has been reviewed extensively (Thomas, 1973; King & Mainwaring, 1974; Gorski & Gannon, 1976). It is currently believed that the steroid penetrates the cell membrane and is bound tightly but reversibly to a cytoplasmic receptor protein. The activated complex moves to the nucleus and gene expression is modified.

The early ontogenetic studies of androgen and oestrogen receptors in various tissues of the rat have been reviewed (Eisenfeld, 1972). In summary, cytoplasmic oestrogen receptors appear to be present in rat uterus and pituitary from the earliest age studied (Day 1 after birth). The uterine receptor is apparently independent of oestrogens since its development until Day 10 was unaffected by ovariectomy. The adult hypothalamic oestrogen receptor appears between Days 12 and 20 of postnatal life and attains adult levels by Day 22 (see also Ginsburg, MacLusky, Morris & Thomas, 1972).

A sharp increase in nuclear bound $[^3H]$oestradiol occurs in the hypothalamus, preoptic area, amygdala and neocortex of female rats at Day 24 when adult levels are attained (Plapinger & McEwen, 1973). The timing of the appearance of nuclear oestrogen correlates well with the disappearance of the plasma oestrogen-binding protein, a-fetoprotein (AFP), from the circulation of developing female rats (Nunez et al., 1971; Puig-Duran, Greenstein & MacKinnon, 1977), and with the rise in free and presumably physiologically active plasma oestradiol (Greenstein, Puig-Duran & MacKinnon, 1977). Kato, Atsumi & Inaba (1974), using sucrose density gradient centrifugation, observed an 8 S peak in cytosols from 7-day-old female rat hypotalami after incubation with $[^3H]$oestradiol. A progressively greater peak occurred until Day 21 and there was a slight reduction at vaginal opening, possibly reflecting competition through increased oestrogen secretion at this time.

Androgen-binding proteins have been found in prostate and seminal vesicle gland cytosols from 20-day-old rats and an adult pattern of $[^3H]$testosterone uptake was apparent in brains of 11-day-old male rats (see Eisenfeld, 1972). Kato (1975) has reported the presence of dihydrotestosterone (DHT)-binding proteins in cytosols from hypothalami of 7-day-old male rats: there was an apparent increase in the numbers of DHT receptors until Day 28 after birth but progesterone receptors could not be detected by the system used. Progesterone receptors have now been demonstrated in cytosols prepared from rat brain and incubated with $[^3H]$progesterone, followed by gel filtration (unpublished). There is evidence for at least two classes of progesterone-binding sites in cytosols from cerebral hemispheres of 10-day-old female rats; one class is of high affinity while the other is of low affinity and apparently unsaturable (Text-fig. 1). Only the latter type of binding could be detected in cytosols from 5-day-old animals. The ontogenesis of the high affinity component is shown in Text-fig. 2. The component is apparent at Day 10, attains a peak concentration in cytosols from 21-day-old rats and falls to adult levels by Day 45.
Text-fig. 1. Scatchard plots of progesterone binding in cytosols from brains of 5- (○) or 10- (○) day-old female rats.

Text-fig. 2. Ontogenesis of high-affinity progesterone binding (mean ± s.e.m., no. of observations in parentheses) in cytosols from brains of developing female rats.

An androgen 8 S receptor has been described in rat epididymal cytosol at Day 20 but not at Day 10 (Calandra, Podesta, Rivarola & Blaquier, 1974). At Day 20 there was a concomitant rise in plasma DHT concentrations, and Calandra et al. (1974) suggested that the synthesis of the epididymal androgen receptor might be under androgenic control. On the other hand, androgen appears not to be required for the maintenance of androgen-receptor activity in the adult ventral prostate (Sullivan & Strott, 1973) or in the brain (Ginsburg & Greenstein, 1976).

Perhaps a useful model for the study of androgen receptors in relation to puberty in the male rat is provided by Roy, Milin & McMinn (1974). They correlated the hepatic output of an androgen-dependent globulin and hepatic androgen-receptor activity: the protein was not excreted in the urine of immature or 'senile' rats and these animals also lacked the receptor. Orchidectomy of the adult reduced the protein excretion and androgen-receptor activity, and both were restored by testosterone treatment.
Testosterone of testicular origin has an apparently organizing influence in brains of neonatal male rats in that it may imprint an acyclic pattern of gonadotrophin secretion from the pituitary of the adult (see Flerko, 1971; Gorski, 1971). It is not surprising, therefore, that attempts were made to identify testosterone receptors in the brains of 2- to 5-day-old rats, but these were unsuccessful (Sherratt, Exley & Rogers, 1969; Barnea, Weinstein & Lindner, 1972). Oestradiol appears to be more potent than testosterone in 'masculinizing' the female brain (see Gorski, 1971). The neonatal rat diencephalon can convert \[^{3}H\]testosterone to tritiated oestrogens in vitro (Naftolin et al., 1975) and in vivo (Weisz & Gibbs, 1974). Furthermore, DHT, a potent androgen which cannot be aromatized, cannot mimic this action of testosterone (McDonald, 1970; Whalen & Luttge, 1971; Arai, 1972). Attention was then focused on the possible existence of oestrogen receptors in the brains of newborn rats. These studies proved more fruitful.

Barley, Ginsburg, Greenstein, MacLusky & Thomas (1974) identified specific oestrogen-binding proteins which were widely distributed in the brains of neonatal rats of both sexes. Specific retention of \[^{3}H\]oestradiol in the diencephalon and limbic system of 2-day-old male rats was demonstrated by autoradiography (Sheridan, Sar & Stumpf, 1974). These observations were confirmed by McEwen, Plapinger, Chaptal, Gerlach & Wallach (1975) who also used tritiated diethylstilboestrol (DES) and \[^{11}B\]-methoxyestradiol (RU2858) which are not bound by AFP. They found labelling in the cortex as well. The receptor has been partly purified by sucrose density gradient centrifugation and it sediments as a salt-dissociable 8 S macromolecule (Westley, Thomas, Salaman, Knight & Barley, 1976).

Further support for the theory of hypothalamic aromatization of testosterone was provided by Westley & Salaman (1976) who used an oestradiol-exchange assay to demonstrate nuclear uptake of the hormone in a widely distributed of 5-day-old rats which included the hypothalamus, but not from the remainder of the brain which showed nuclear exchange of \[^{3}H\]oestradiol after the in vivo administration of DES but not testosterone. Westley & Salaman (1976) proposed that nuclear binding of oestrogen after testosterone administration occurs only where the aromatase systems are present.

The disappearance of the rat neonatal cortical oestrogen receptor between Days 10 and 20 of postnatal life (McEwen et al., 1975) is suggestive of a developing inhibitory influence on neural oestrogen-receptor synthesis, resulting in the ultimate concentration of sites within specific hypothalamic, preoptic, limbic and mid-brain areas (Stumpf, 1968; Pfaff, 1968; Stumpf & Sar, 1971). This inhibitory influence does not appear to be modified in the female rat brain by neonatal administration of testosterone (see Eisenfeld, 1972; Maurer, 1974; Maurer & Woolley, 1975). There may be, however, a reduced androgen-receptor population in the pituitary of adult male rats treated neonatally with oestradiol (Simmons, 1971).

There are no reports of marked changes in neural or pituitary receptor concentrations at the perinatal period, except for that of Monbon, Loras, Reboud & Bertrand (1974), who found an apparent increase in pituitary retention of \[^{3}H\]testosterone after administration of the tritiated hormone to orchidectomized rats between 6 and 9 weeks of age.

The ontogenesis of receptors for other steroids is virtually unexplored, apart from the development of glucocorticoid receptors in rat liver (Giannopoulos, 1975) and rat intestine (Henning, Ballard & Kretchmer, 1975). In this respect the ovary urgently requires investigation. Schreiber, Reid & Ross (1976) have reported the presence of an 8 S androgen-binding protein in cytosols prepared from ovaries of oestrogen-stimulated, hypophysectomized immature rats.

In summary, receptors for the sex steroids appear to be present well before the onset of puberty. It is unlikely that any sudden change in steroid-receptor activity precipitates its onset, and the long-held view that the initiation of cyclic gonadotrophin secretion and regular ovulation occurs as a result of altered neural sensitivity to circulating oestrogens (see Grumbach, Roth, Kaplan & Kelch, 1974) apparently cannot be explained in terms of steroid receptors.

**Gonadotrophin receptors**

A principal site of action of the gonadotrophins appears to be the cell membrane, where they combine with specific receptor sites known to activate the membrane-bound enzyme, adenylate cyclase;
cyclic AMP production is modified with consequent effects on the protein kinases. Protein synthesis de novo may also occur (for reviews see Butcher, Robison & Sutherland, 1970; Catt & Dufau, 1976; Ryan & Lee, 1976). In contrast to studies of steroid hormone receptors, those carried out of gonadotrophin receptors in the developing rat and pig ovary hold more promise of providing a plausible explanation for the first luteinization.

The ovary

Presl, Pospíšil, Figarová & Wagner (1972) injected $^{125}$I-labelled hCG (rather than the less stable LH) into female rats of 5–30 days of age and, 24 h later, prepared autoradiographs of ovarian and pituitary tissue. Labelling was confined mainly to theca and interstitial cells of the ovary and was low in the granulosa cells. In the former cell types labelling increased in density over Days 8 and 9 and in the pituitary from Days 5 to 10. Goldenberg, Vaitukaitis & Ross (1972) injected DES into hypophysectomized 23-day-old female rats and observed an increased saturable uptake of $[^3H]$FSH by the ovary, and a stimulation of $[^3H]$thymidine incorporation into granulosa cell DNA. FSH promoted antrum formation after DES priming and DES was more effective than FSH in promoting granulosa cell proliferation. Goldenberg et al. (1972) suggested that oestrogens increase the numbers of FSH-responsive cells and the numbers of follicles responsive to FSH. Endogenous oestrogen may be important in the development of the granulosa cells, since administration of an oestrogen antibody to rats produced a fall in ovarian weight and a reduction in maximum follicle diameter, even at 2–5 days of postnatal life (Reiter, Goldenberg, Vaitukaitis & Ross, 1972). Administered oestrogen, however, does not appear to increase the numbers of FSH receptors per cell, although the hormone does cause a proliferation of granulosa cells and therefore an increase in the number of receptors per ovary (Louvet & Vaitukaitis, 1976; see also Goldenberg et al., 1972).

Evidence is accumulating that FSH stimulates the synthesis of its own receptors and of LH receptors also. Zeleznik, Midgley & Reichert (1974) injected FSH, hCG and DES into 25-day-old rats and prepared autoradiographs from the ovaries after topical application of $^{125}$I-labelled hCG or FSH. The activity of 3β-hydroxysteroid dehydrogenase was localized and binding of the radioactive hCG to isolated granulosa cells was measured. In ovaries from saline-treated animals, $^{125}$I-labelled hCG was bound to theca and interstitial cells and the enzyme was predominantly in the same cell types. But after 2 days of FSH treatment, $^{125}$I-labelled hCG was bound to granulosa cells as well, and 3β-hydroxysteroid dehydrogenase activity was also present; hCG and DES were without effect.

LH receptors have been measured and partly characterized on porcine granulosa cells (Channing & Kammerman, 1973). Kammerman & Ross (1975) measured LH receptors on isolated porcine granulosa cells and found a 35-fold increase in receptors per cell as the follicle enlarged from 1–2 mm to 6–12 mm, although granulosa cell size was unchanged. Similar results were obtained by Lee (1976) who also measured increased cyclic AMP activity with maturation of the follicle. More recently, Nimrod, Tsafiriri & Lindner (1977) reported that fragments from ovaries of 28- to 30-day-old DES-treated rats incubated in vitro with FSH exhibited a 5-fold increase in saturable binding of hCG over control incubates, although the effect could not be shown when isolated granulosa cells were used. Presumably an essential ingredient is provided by the thecal or interstitial cells or both.

An FSH-specific receptor on rat granulosa cells has been characterized in vitro (Nimrod, Erickson & Ryan, 1976). The effects of exogenously applied FSH on its own receptor are of particular interest. As the follicle matures it is exposed to constantly changing ratios of oestrogen, FSH and LH. It is possible that the timing of exposure and the state of the follicle dictate its response. This possibility was investigated by Richards et al. (1976) with 24-day-old hypophysectomized female rats injected with a rather high dose of oestradiol (2 mg/day), followed by FSH and LH in spaced dosage regimens. They found complex effects. Oestrogen alone had a stimulant effect on its own receptors and a depleting effect on granulosa cell LH receptors. Oestrogen followed by FSH + oestrogen resulted in an increase in both receptor types. Although oestrogen was required for the synergistic effect on LH receptors, the effect on FSH receptors could be achieved without oestrogen. The same regimen followed by an LH injection resulted in a fall in LH and FSH receptors for 48 h afterwards, and LH caused luteinization. But this effect of LH on receptors was also achieved with saline alone. The fall in receptor numbers after saline presumably reflects the actions of oestrogen and FSH in combination.
Several interpretations of these observations have been advanced (Richards & Midgley, 1976). In essence, it is suggested that there is an interaction between granulosa and thecal cells under the influence of oestradiol and FSH. The net result may be the stimulation of sufficient LH receptors on the follicles to cause luteinization of those which are at a critical stage of development. Atresia will result if the follicle loses receptors for oestradiol, FSH and LH. This concept, while awaiting further study, is nevertheless attractive and could form the basis for an understanding of the events preceding the first ovulation. For example, the initial oestrogen stimulus in the developing female rat could originate from the appearance in the circulation at Day 21 to 23 of free and therefore physiologically active oestrogen (Greenstein et al., 1977). About 19 days are required for the development of the follicle from oocyte to the antral stage (see Richards & Midgley, 1976) and the first ovulation will therefore occur at about 35-40 days of age. The interval between the appearance of free oestrogen and the first ovulation is thus due to the development of follicles under the influence of a balanced milieu of oestrogen and FSH to provide the necessary population of LH receptors for ovulation. The delay could also be due to the organization of neural rhythms necessary for the first surge of LH (see MacKinnon, Puig-Duran & Layne, 1978). Subsequent ovulations are then controlled by the established neural rhythms and the waves of follicle cohorts which have attained critical numbers of LH receptors at the time of the cyclic LH surge. It is interesting to note that in ovaries of 21-day-old rats 125I-labelled FSH is bound only to granulosa cells and LH receptors are confined to thecal and interstitial cells, but at 33 days of age LH receptors are present on granulosa cells as well (Peluso, Steger & Hafez, 1976). The above hypothesis, which is put forward solely for heuristic purposes, dispenses with the need for altered CNS sensitivity to ovarian hormones at puberty. Moreover, vaginal opening in rats has been advanced by only 3 days after ovary autotransplantation (see Donovan & van der Werff ten Bosch, 1965).

The testis

The testis of the developing rat contains receptors for both LH and FSH. De Kretser, Catt, Burger & Smith (1969) localized binding of 125I-labelled LH on interstitial cells of the testis of immature rats and in the proximal convoluted tubule of the kidney by autoradiography. Catt, Dufau & Tsuruhara (1971) used the binding of radiiodinated LH and hCG to interstitial cells of the rat testis to develop a radioligand receptor assay for LH and hCG. In an autoradiographic study, de Kretser, Catt & Paulsen (1971) raised the possibility of cytoplasmic binding of 125I-labelled LH in rat testis interstitial cells, but this appears to be an isolated finding. Means & Vaitukaitis (1972) showed that the tubular tissue of rat testis binds [3H]FSH specifically. The dissociation constant \( K_d \) of the binding reaction was \( 7 \times 10^{-9} \) mol/l and the binding, which was present predominantly on the membrane fraction, was abolished by a protease. Very little [3H]FSH was bound to interstitial tissue.

In an ontogenetic study of 125I-labelled LH binding to testis homogenates from rats of 5, 22 or 130 days of age, Sharpe, Hartog, Ellwood & Brown (1973) were able to detect saturable, specific binding of the radioligand only in homogenates from 130-day-old rats. This type of binding was present in homogenates from 22-day-old animals in negligible amounts. This is not surprising, because in 20-day-old rats Leydig cell number is very low, and cells proliferate rapidly thereafter (Knorr, Vanha-Perttula & Lipsett, 1970). Leydig cell numbers plateaued at 40 to 50 days of age and fell thereafter, but testosterone secretion increased sharply from 40 to 60 days. Clegg (1966) had already shown that there is an exponential fall in rat Leydig cell growth increments after 52 days of age. A depletion of interstitial cell lipid droplets between Days 45 and 51 also correlates well with the increased testosterone secretion at this time (Knorr et al., 1970). According to Lording & de Kretser (1972) the numbers of interstitial cell lipid droplets in the rat testis fell during the first 2 weeks of postnatal life and then disappeared. The results described above are suggestive of increased Leydig cell sensitivity to circulating LH at puberty. On the other hand, Frowein & Engel (1975) reported that Leydig cell-enriched fractions from 30-day-old rat testes bound 125I-labelled hCG to the same extent as those from adults.

The binding of FSH to rat testicular tissue in vitro increased from 5 to 15 days and remained at the 15-day value thereafter (Desjardins, Zeleznik, Midgley & Reichert, 1974), while 125I-labelled
LH or hCG binding increased from 5 to 55 days and the numbers of binding sites per cell remained constant thereafter. Desjardins et al. (1974) also localized (autoradiographically) radioactive hCG over interstitial cells and FSH over seminiferous tubules. Steinberger, Thanki & Siegal (1974) measured binding of $^{125}$I-labelled FSH in testis homogenates. The $K_d$ of the binding reaction was $1.6 \times 10^{-9} \text{ mol/l}$, a lower value than that measured by Means & Vaitukaitis (1972). More hormone was bound in homogenates from 10- to 25-day-old rats than from older animals.

The functional significance of FSH binding in rat testis was investigated by Means, Fakunding, Huckins, Tindall & Vitale (1976), who used irradiated rats which possess apparently normal Sertoli and Leydig cells but no germ cells. FSH receptors appeared to be confined to the Sertoli cell. FSH stimulated RNA polymerase activity and protein synthesis in Sertoli cell-enriched extracts. Under the influence of FSH there was an increase in the production of the androgen-binding protein (ABP) by these extracts. ABP production was also stimulated by 8-bromocyclic AMP.

It is possible that the membrane-bound receptors are differentiated early in the testis, and perhaps coincidentally with the formation of the cell. They are certainly present during intrauterine life. LH receptors have been detected in rat testis at 20 days of gestation and in man during the 26th week of fetal life (Frowein & Engel, 1974). Testosterone is present in the testis of the 10-week-old human fetus in fairly high concentrations (1400 pg/ml; Reyes, Winter & Faiman, 1973). In the fetal rabbit gonad, [$^3$H]testosterone production from [$^3$H]pregnenolone can be shown on Day 19, immediately before irreversible sexual differentiation (Wilson & Siiteri, 1973). At Day 17 of fetal life [$^3$H]dehydroepiandrosterone is formed, but not testosterone. Since the Leydig cells are already present at Day 17 (Wilson & Siiteri, 1973), it is likely that the LH receptor is functional when the cell is differentiated, but the intracellular 3β-hydroxysteroid dehydrogenase–isomerase enzymes become active on Day 19. Catt, Dufau, Neaves, Walsh & Wilson (1975) measured a sharp increase in binding of $^{125}$I-labelled hCG to rabbit testis homogenates coinciding with an increase in homogenate testosterone concentration between 17 and 19 days of age.

The immature mammalian testis may be fully equipped with the hormone receptor populations required for normal spermatogenesis and testosterone formation, but all the necessary cell enzyme systems may not be active when the cell is differentiated.

The author is a fellow of the Mental Health Foundation.

References


Hormone receptors


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