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Natural and anthropogenic environmental oestrogens: the scientific basis for risk assessment*

Comparative physiology of the reproductive endocrine system in laboratory rodents and humans

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INTRODUCTION

Many aspects of reproduction are controlled by hormones, which serve as chemical messengers linking together the various reproductive organs to form an integrated and precisely regulated homeostatic system. Any disruption of this balanced system can lead to inappropriate development, maintenance and function of reproductive activity, resulting in some cases in mild or severe infertility. It is therefore of little surprise that there should be such concern over the potential for chemicals to have adverse reproductive health effects by acting as endocrine disrupters. This chapter will begin with an overview of the basic components and function of the reproductive system and will be followed by a more detailed consideration of the endocrine control of male and female reproductive processes. It is intended that this chapter will provide a broad understanding of how the reproductive endocrine system functions, in order to understand some of the potential mechanisms by which endocrine disrupters might have their effects. Key species differences will be highlighted for humans and laboratory animals throughout the chapter.

OVERVIEW OF REPRODUCTIVE ENDOCRINE SYSTEM

An organisational overview of the individual components of the reproductive endocrine system is presented in Fig. 1. The hormonal control of reproduction begins in the brain. The hypothalamus through the secretion of gonadotrophin-releasing hormone (GnRH) governs the activity of the pituitary gland, an organ which serves as an amplifier, transmitting the brain signal via the secretion of the gonadotrophins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), to act on the gonads. In the female, LH and FSH stimulate the processes of folliculogenesis and ovulation, and cause the follicles and corpus luteum to secrete oestrogens and progestagens. In the male LH and FSH control the process of spermatogenesis and stimulate the secretion of testosterone from the Leydig cells. The sex-steroids have a wide variety of physiological effects, acting on a number of different targets in the body (see below). The reproductive endocrine system can also be controlled by external signals such as changes in daylength, or olfactory signals in the form of pheromones, which appear to influence the way that the central nervous system controls the secretion of GnRH.

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Anatomical organisation of the hypothalamus and pituitary gland

Hypothalamus

The hypothalamus forms part of the diencephalon, a subdivision of the forebrain, and lies immediately below the thalamus. It surrounds the third ventricle and is bounded rostrally by the optic chiasma, and caudally by the mamillary bodies. Many of the cell bodies of neurones within the hypothalamus are found in specific nuclei. For instance, neurones containing corticotrophin-releasing factor (CRF) which stimulates the secretion of adrenocorticotrophin (ACTH) from the pituitary gland, are found within a specialised structure called the paraventricular nucleus (1). In contrast, neurones containing GnRH have a much more diffuse distribution throughout a larger hypothalamic region called the pre-optic area (2). Studies in the mouse, rhesus monkey and human have shown that GnRH neurones do not begin their life in the central nervous system (3–5). Instead, they arise during early embryogenesis from the olfactory placode and migrate along the pathway of the nervus terminalis-vermonasal complex to their resting place in the pre-optic area. In mice, GnRH neurones are first detected in the olfactory placode by embryonic day 9.5 (e9.5), after which time there is a period of rapid mitosis with all cells that make up the postnatal population being present by day 12.5. Migration into the CNS takes place after e12.5 and is complete by e16.5 (4). In humans with Kallmans Syndrome (hypogonadotrophic hypogonadism coupled with anosmia), there are no detectable GnRH neurones in the hypothalamus (3). Instead GnRH neurones remain in their embryonic birthplace in the olfactory placode. This lack of migration appears to result from a deficiency in neural cell adhesion molecule which has been shown to form a scaffold through which the GnRH neurones pass (6). Axons derived from cell bodies located in the various nuclei of the hypothalamus, project to a number of other parts of the brain, including the hypothalamus and the median eminence, or to the posterior lobe of the pituitary gland. Once GnRH cell bodies have migrated to the pre-optic area, axons are then targeted to the lateral median eminence, which is located at the base of the hypothalamus and is the anatomical link between the neural and endocrine systems. Neurones projecting to this region form terminal connections with a capillary plexus which feeds into the hypothalamo-hypophyseal portal blood supply. This blood supply consists of portal vessels which course down the pituitary stalk and provide the humoral link between the hypothalamus and the pituitary gland (see Fig. 1). The mechanisms responsible for the migration and functional organisation of the GnRH neuronal system are poorly understood but are thought to involve locally produced growth factors, called neurotrophins, which influence neural survival and target cell recognition (7). The production of these growth factors is known to be regulated in part by steroid hormones such as oestrogens (8, 9), but the potential effects if any, of xenobiotic oestrogens on this aspect of developmental neuroendocrinology is not known. However, if xenoestrogens should act to alter the neuroendocrine organization of the GnRH neuronal system during development, this could have long-term adverse consequences for reproductive endocrine function.

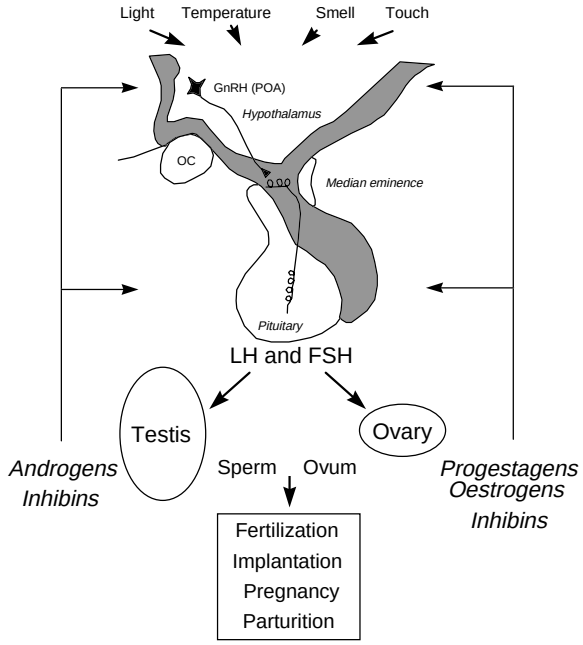

Fig. 1. Organisational overview of the hypothalamo-pituitary gonadal axis.

Pituitary gland

The pituitary gland comprises two main lobes, the anterior lobe and the posterior lobe. The posterior lobe consists mainly of neural tissue and is linked directly with the hypothalamus by neural fibres whose cell bodies are located within the supraoptic and paraventricular nucleus of the hypothalamus. The main hormonal products of the posterior lobe are oxytocin, which is responsible for the ejection of milk from the mammary gland and for uterine contractility at birth, and arginine vasopressin which is responsible for controlling blood volume.

The anterior lobe is further subdivided into three regions, the pars distalis, pars intermedia, and pars tuberalis. The anterior lobe is largely devoid of nerve fibres, apart from sparse innervation of the pars intermedia. It is instead, linked to the hypothalamus by the hypophyseal-pituitary portal blood supply. The anterior pituitary gland synthesises and secretes six individual hormones, adrenocorticotrophin (ACTH) growth hormone (GH), prolactin (PRL), thyroid stimulating hormone (TSH) luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH and FSH are found within one cell type, the gonadotroph, whereas the remaining hormones are synthesised within individual cells; ACTH in corticotrophs, GH in sommatotrophs, PRL in lactotrophs and TSH in thyrotrophs (see Fig. 2). During early embryogenesis the five specialised cell types of the anterior pituitary gland are derived from a common progenitor cell, but there is a spatial and temporal pattern to the appearance of each of these (10). Corticotrophs are the first cell types to appear, followed by thyrotrophs, gonadotrophs and finally lactotrophs and somatotrophs. A number of homeodomain factors (e.g. Pit 1, Lhx3) have recently been described which specify the appearance of these cell types (10,11). Each anterior pituitary hormone is controlled by stimulatory or inhibitory factors which are released from the hypothalamus (see Fig. 2). For the gonadotrophins there is a single releasing factor, GnRH, although for many years there has been a great deal of unresolved controversy as the existence of a separate FSH releasing factor (12). ACTH is under multifactorial control, with CRF and AVP being the main releasing factors and oxytocin and norepinephrine having minor stimulatory roles by comparison. Growth hormone is stimulated by growth hormone releasing factor (GRF) and inhibited by somatostatin (SRIF). Thyroid stimulating hormone is stimulated by thyroid hormone releasing factor (TRH). Prolactin is primarily under inhibitory control by dopamine, but in rats there is also evidence for the existence of a prolactin releasing factor (13). This factor which is of unknown identity originates from the intermediate and posterior lobes of the pituitary gland and is responsible for mediating some of the stimulatory effects of oestrogen on prolactin secretion

(14). The xenobiotic oestrogen, bisphenol A has recently been shown to stimulate prolactin secretion in rats via the release of this prolactin releasing factor, illustrating the importance of this pathway in any consideration of the effects of endocrine disrupters on prolactin secretion (15). The control of prolactin secretion is further complicated by the presence of two peptides, galanin and vasoactive intestinal peptide (VIP), in the anterior pituitary gland (16). Galanin and VIP stimulate prolactin secretion and are themselves inhibited by prolactin and stimulated by oestrogen (16). It is proposed that the local production of these peptides in the anterior pituitary gland serves as a way of mediating the effects of oestrogens on the secretion of prolactin, and as such this represents another potential pathway by which prolactin may be controlled by xenoestrogens.

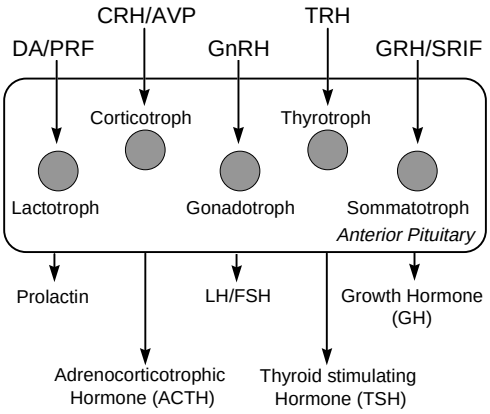

Fig. 2. Hypothalamic control of the pituitary gland illustrating the five hormone secreting cell types present in the anterior pituitary gland, and their control by hypothalamic stimulatory and inhibitory factors.

Gonadotrophin releasing hormone (GnRH)

GnRH is a decapeptide which is derived from a larger precursor molecule called prepro-GnRH. Until recently, it was thought that the majority of mammals had only one form of GnRH in the brain. This contrasts distinctly with birds and amphibians which have at least two forms (see pp. 1657–1669). However, a second form of GnRH has been isolated from stumptail and rhesus monkeys which was similar in structure to the second form of GnRH found in chickens (cGnRH-II). The functional significance of this second form of GnRH remains to be determined (17). The regulation of GnRH synthesis and secretion involves a number of inhibitory and stimulatory neurotransmitters and neuropeptides located within the central nervous system and this is considered in detail in pp. 1647–56.

GnRH stimulates the synthesis and secretion of LH and FSH in the pituitary gonadotrophs by binding to a specific cell surface receptor which is part of a family of receptors collectively called G-protein coupled receptors (GPCRs). GnRH receptors have been cloned from a variety of species and show structural and sequence homology (18–20). GnRH stimulation of this receptor causes activation of the phopholipase C signal transduction pathway, which is induced by g-protein and leads to mobilisation of calcium and activation of protein kinase C (21). GnRH receptor expression is regulated by GnRH itself but also by oestrogen (22). In rats, ovariectomy increases pituitary GnRH receptor mRNA levels and this is reversed by treatment with oestrogen. It is not known if xenobiotic oestrogens can alter GnRH receptor expression, but this could clearly be an important point of regulation.

Gonadotrophins

LH and FSH are glycoprotein hormones composed of two dissimilar subunits, the alpha subunit and the beta subunit, which are linked by disulphide bonds. The alpha subunit is shared by LH and FSH and also by the other glycoprotein hormones, TSH and chorionic gonadotrophin (found in humans and equids only). Each glycoprotein hormone has a hormone specific beta subunit which is encoded in a separate gene and is responsible for the biological activity of the hormone. The degree of glycosylation of the

individual gonadotrophin subunits alters the circulatory half-life of each of the gonadotrophins and can also alter their biological activity. This is thought to be an important mechanism for controlling the physiological activity of the gonadotrophins at different stages of the reproductive cycle.

In females FSH is responsible for follicular growth and LH stimulates sex-steroid synthesis and secretion. In males FSH stimulates Sertoli cell replication and function whereas LH acts on the Leydig cells to control testosterone synthesis and secretion. LH and FSH exert their effects on target cells by interacting with a cell surface LH or FSH receptor (23). These receptors consist of a large extracellular domain which is responsible for hormone binding, seven transmembrane regions and an intracytoplasmic tail. Different species share the same basic receptor structure, but with some differences in amino acid make up of the polypeptide chain. Overall the human and rat LH receptor share 85% homology, but despite this the human receptor has a high degree of specificity, and will not bind rat LH. The actions of LH and FSH via these receptors is mediated by the cAMP second messenger system which is induced by adenylate cyclase and g-proteins (24).

A distinguishing feature of gonadotrophin (and other anterior pituitary hormones) secretion, is its inherent pulsatility. This is particularly so for LH which is secreted as discrete ultradian bursts whereas FSH exhibits a more constant pattern with fluctuations which cannot be easily defined as true hormone pulses. However, both gonadotrophins are under the control of GnRH which itself is secreted in a pulsatile fashion from the hypothalamus. The differences in the circulating patterns of LH and FSH are primarily accounted for by differences in the circulatory half lives of the two hormones. LH has a half life of approximately 20 minutes whereas FSH is about 3 hours. The frequency at which gonadotrophin pulses occurs is dictated to a large extent by the concentrations of sex-steroids in the circulation through a mechanism known as negative feedback. Gonadotrophins stimulate the secretion of sex-steroids from the gonads and these in turn have a negative feedback effect on the hypothalamo-pituitary unit to inhibit gonadotrophin secretion. Sex-steroids exert these negative feedback effects by acting on the hypothalamus and the pituitary gland. The pituitary possesses many receptors for sex-steroids and is well known to be a major site of steroid negative feedback, largely by reducing the sensitivity of the pituitary gland to the incoming GnRH signal (25, 26). Negative feedback on the hypothalamus is also well established, but surprisingly, GnRH neurones do not possess steroid receptors to mediate this effect (27). Instead it is thought that steroids modulate the activity of other neurones such as those containing the inhibitory amino-acid neurotransmitter, γ-aminobutyric acid (GABA) which in turn controls the activity of the GnRH neurone (28). This negative feedback system is exquisitely sensitive and maintains homeostasis within the reproductive system. Steroid hormones are not the only negative feedback regulators of gonadotrophins. Inhibin, is a glycoprotein hormone secreted from the granulosa cells of the follicle and the Sertoli cells in the testis (see the section on Inhibins and activins) which specifically inhibits the secretion of FSH. Inhibin can either act alone or in concert with sex steroids to regulate FSH secretion. Thus, the role of inhibin in the control of FSH secretion varies between genders, and may also be species specific. For instance, injection of inhibin antiserum fails to elevate FSH secretion in adult male rats but results in a significant elevation of FSH in the Rhesus monkey (for review see (29)). One of the main pathways by which xenoestrogens may act as endocrine disruptors is thought to be by interfering with these negative feedback pathways resulting in suppressed circulating concentrations of LH and FSH (see below).

The preovulatory surge of gonadotrophins occurs in females as a result of a switch in the responsiveness of the hypothalamus and pituitary gland to allow oestrogen to have a positive, rather than negative feedback effect. This allows oestrogen secreted from the dominant follicle, to stimulate LH and FSH thereby causing ovulation of that follicle. During the preovulatory surge LH and FSH concentrations can reach values some 10–20 times higher than seen at other times of the cycle. In rhesus monkeys the major site for positive feedback effects of oestrogen appears to be the pituitary gland. Surgical lesions of the mediobasal hypothalamus reduce plasma LH and FSH concentrations. These can be restored by administering pulses of GnRH, and in this situation oestrogen is able to elicit a gonadotrophin surge (30). Clearly this must be due to a direct effect on the pituitary gland because the hypothalamus has been destroyed. The same mechanism is also thought to occur in women. However, positive feedback can also occur at the hypothalamus as has been demonstrated by a series of experiments in which GnRH secretion from the hypothalamus has been measured and shown to increase

dramatically during the gonadotrophin surge in monkeys (31), sheep (32) and rats (33). Oestrogen therefore appears to be able to exert its positive feedback actions at the level of the hypothalamus and the pituitary gland.

In rats, the LH surge is coupled to the light-dark cycle and always occurs during the late afternoon; there is little evidence for a similar link in humans. In rats the gonadotrophin surge is also sexually differentiated. Sexual differentiation of the central nervous system is mainly affected by gonadal steroids which induce permanent and irreversible effects on the structure and function of specific regions in the brain (34). These organisational effects of sex-steroids can only occur during critical windows of development. Early exposure to androgens secreted from the developing testes, results in the abolition of cyclic female patterns of gonadotrophin secretion. These animals can no longer produce the LH surge which is characteristic of ovulation in females. Paradoxically, in rats oestrogen is able to mimic the effects of androgens and this led to the aromatization hypothesis (34). The brain possesses aromatase enzyme during foetal and neonatal development which is capable of converting testosterone into oestradiol (see Fig. 3). It is this local production of oestrogen in the pre-optic area of the hypothalamus of the male brain which induces sexual differentiation of the surge mode of gonadotrophin secretion in rats. The critical window for sexual differentiation of the brain varies between species (rats day 18–27 post conception; mouse, postnatal; guinea pig, 30–37 days post conception). Certain aspects of sexual behaviour are also sexually differentiated. For instance, androgenic hormones given either pre- or post natally to developing female rats decreases the rate of lordosis behaviour (35). The sexually dimorphic nucleus of the pre-optic area of the hypothalamus (SDN-POA) is a region believed to be involved in the control of sexual behaviour, and is larger in volume in males compared with females (36). Recent studies have shown that administration of phytoestrogens such as genestein during the critical window, can increase the volume of the SDN-POA of female offspring to that seen in males (37). Administration of the xenobiotic oestrogen, octylphenol, was found to have no effect on this region, despite having clear oestrogenic effects to increase uterine weight (38). The effects of individual xenobiotic oestrogens on sexual differentiation of CNS function therefore remain to be clarified. In humans there is considerable controversy as to the extent to which sexual behaviour is sexually differentiated (39). There is however, little evidence for sexual differentiation of the surge mode of gonadotrophin secretion. Oestrogen administration to men is able to elicit a normal gonadotrophin surge.

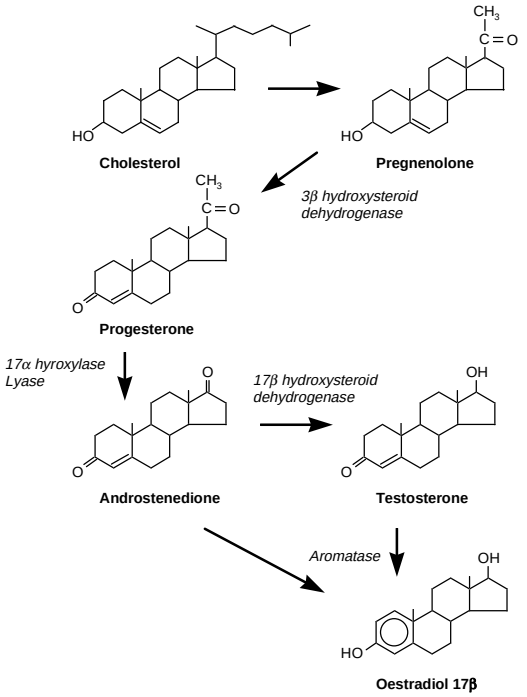

Fig. 3. Simplified biosynthetic pathway illustrating the main enzyme steps involved in the production of the common sex-steroids from cholesterol precursor.

Sex steroids

The steroid hormones comprise a large group of compounds which are all derived from the precursor cholesterol. The pathway for the synthesis of the main sex-steroids is shown in Fig. 3. The conversion of cholesterol to pregnenolone represents the first step in the pathway for steroid biosynthesis. This is an important rate limiting step which is common to all steroids and takes place on the inner mitochondrial membrane. Until recently, little was known about the way in which cholesterol gains access to the inner mitochondrial membrane but a new protein called steroid acute regulatory protein (StAR) has now been identified whose function is to facilitate the transport of cholesterol from the outer to the inner mitochondrial membrane (40). There are three main groups of sex-steroids, the androgens, progestagens, oestrogens. They are all related structurally and are formed by the actions of a series of enzymes which form the steroidogenic pathway. In brief this pathway causes the conversion of pregnenolone to progesterone which is the precursors for androgens. Androgens can then be converted into oestrogens. The extent to which these conversions take place is dependent on the expression of the various enzymes in specific tissues. For instance, tissues which lack the enzyme aromatase are unable to convert androgens to oestrogens and are therefore not a site of oestrogen production. The majority of steroids do not circulate in blood as free hormone, but instead are bound to a variety of serum proteins. Most oestrogen in the circulation is bound to sex-hormone-binding globulin (SHBG), but compounds such as ethinyl oestradiol and DES do not bind to SHBG (41). Many environmental oestrogens also fail to bind to SHBG, and this could increase their bioavailability in the circulation.

Sex steroids have a wide range of effects in many organs and tissues throughout the body. Androgens are essential for normal spermatogenesis but also maintain the normal integrity of the male secondary sex organs such as the prostate, seminal vesicles, and epididymes. Androgens also influence male sexual and aggressive behaviour, and act on the hypothalamus and pituitary gland to control gonadotrophin secretion. Oestrogens have a major influence on uterine physiology. Removal of the major source of oestrogen by ovariectomy, causes marked atrophy of the uterus. This can be restored by administration of exogenous oestrogen, which causes generalised oedema and stromal cell proliferation in the endometrium. This uterotrophic effect of oestrogen forms the basis of the uterotrophic assay which is a sensitive means of assessing the oestrogenic properties of natural and xenobiotic oestrogens (42). Oestrogens also regulate female sexual behaviour and secondary sexual characteristics. They participate in the development of the ovum and prepare the uterine endometrium for progesterone action, by inducing progesterone receptors. They sensitize the uterine myometrium at birth and are important for mammary gland development. Oestrogens regulate gonadotrophin secretion and are responsible for some aspect of sexual differentiation of the central nervous system. Oestrogens also have many non-reproductive effects on bone, the cardiovascular system, and lipid metabolism. Progestagens are important for the maintenance of pregnancy, by dampening down the contractile activity of the myometrium. They prepare the uterine endometrium to receive the developing conceptus, and are involved in mammary gland development. They also regulate gonadotrophin secretion.

In order for steroids to exert these effects in target tissues they must interact with an intracellular receptor belonging to the superfamily of nuclear hormone receptors (43, 44). Each class of steroid hormone has a specific steroid receptor, but they all, have certain structural features in common. In particular all steroid receptors have two specialised regions called the ligand binding domain (LBD) and the DNA binding domain (DBD). The LBD is the region where the steroid hormone binds the receptor and the DBD is the region which allows the receptor to interact with the promoter region of target genes. In their inactivated state these receptors are located in the cytoplasm of cells, but once bound by steroid hormone (which passes into the cell by passive diffusion) they move into the nucleus. These activated receptors then dimerize and bind to specific sequences on oestrogen responsive genes called oestrogen response elements, to initiate the transcriptional process (43, 44).

For oestrogens, there are known to be two receptors each encoded on a separate gene, called ERα and ERβ. Structurally, ERβ is highly homologous to ERα in the DNA binding domain (>95% amino-acid identity) but shows only 55% homology in the ligand binding domain (45, 46). These structural differences lead to different relative binding affinities in ligand binding assays, of a number of oestrogenic ligands including xenoestrogens such as methoxychlor and bisphenol A and phyto-oestrogens such as coumestrol and genistein (47). For both groups of compounds the relative binding affinity was greater for ERβ compared with ERα. Furthermore, ERβ shows a discrete tissue distribution being the most predominant ER in the prostate, ovary and certain regions of the brain such as the paraventricular nucleus (45, 47–49). Differential oestrogen receptor subtype distribution may account for the ability of natural and xenobiotic oestrogens to exert tissue specific effects.

Inhibins and activins

Inhibin is a glycoprotein hormone consisting of two dissimilar subunits termed α and β, linked by disulphide bonds, which selectively inhibits the secretion of FSH (50). Two forms of the β-subunit have been isolated, termed β_A and β_B, which combine with the α-subunit to produce inhibin A and inhibin B. In addition dimerisation of the β-subunits results in the formation of activins (activin A; $\beta_A\beta_A$, activin AB; $\beta_A\beta_B$ or activin B; $\beta_B\beta_B$) which have been shown to stimulate the synthesis and secretion of FSH (51). Recent cDNA cloning and sequence analysis from several species has revealed that the three inhibin subunits (α, β_A, β_B) are encoded by three separate genes (52). It is generally accepted that the main sites of production of inhibin reside within the gonads, primarily the Sertoli cells in the testis and the granulosa cells in the ovary. Although one of the primary functions of circulating inhibin is to inhibit FSH secretion it is now clear that inhibins and related peptides can act as paracrine and autocrine factors in other parts of the body, such as the central nervous system, pituitary gland and placenta (53).

ENDOCRINE CONTROL OF THE FEMALE REPRODUCTIVE SYSTEM

Every mammalian species with the exception of humans displays oestrous behaviour at ovulation, and this gives rise to the term oestrous cycle. In humans the only outward sign of cyclicity is menstruation and we therefore refer to the human menstrual cycle. Within these broad categories there are many species differences in the type and length of cycle. Animals can be grouped according to whether they are spontaneous or induced ovulators. Induced ovulators include the rabbit, in which the act of coitus is necessary to induce ovulation. The majority of mammals fall into the category of spontaneous ovulators which do not require the act of coitus to trigger ovulation. However, laboratory rats and mice are spontaneous ovulators but also require the act of coitus to produce a fully functional corpus luteum. Mating stimulates the secretion of prolactin which forms part of the luteotrophic complex and maintains the lifespan of the corpus luteum. Animals can also be classified according to whether their cycles occur at particular times of the year, in which case they are referred to as seasonally oestrous. In the female, xenoestrogens have been shown to have direct effects on target organs such as the uterus and mammary gland, and in the case of the uterus this has lead to the development of a sensitive assay for detecting the oestrogenic activity of chemicals. However, the effects and consequences of xenobiotic oestrogens acting on the endocrine control of the female reproductive system remain largely unknown. The endocrine control of the human, rat and rabbit cycles will now be considered in detail.

Human

The human menstrual cycle can be divided into two phases, the follicular phase and the luteal phase. Throughout the menstrual cycle, primordial follicles undergo gonadotrophin independent follicular growth to form early preantral follicles. In humans only one preantral follicle will be selected to form a dominant preovulatory follicle, a mechanism which is dependent on the secretion of LH and FSH from the pituitary gland (see Fig. 4). Preantral follicles which are not selected undergo a process of atresia and die (54). Following luteolysis, oestrogen, progesterone and inhibin concentrations fall, thus reducing

negative feedback and allowing gonadotrophin concentrations to rise. The rise in LH and FSH stimulates the developing follicles to produce steroids and inhibin. Androgens produced in the thecal cells serve as a substrate for oestrogen synthesis in the granulosa cells (55). Aromatase expression in the granulosa cells is stimulated by androgens and FSH, so as to facilitate oestrogen synthesis (55). In this way a local positive feedback loop is established which causes ever increasing amounts of oestrogen to be produced from the most active follicle, and this culminates in a surge release of oestrogen. The surge in oestrogens triggers the preovulatory surge in LH and FSH by a positive feedback mechanism, and this causes ovulation (30). Oestrogens and FSH also play an important role in inducing LH receptors on granulosa cells, which is a crucial step in the process of ovulation (56) .

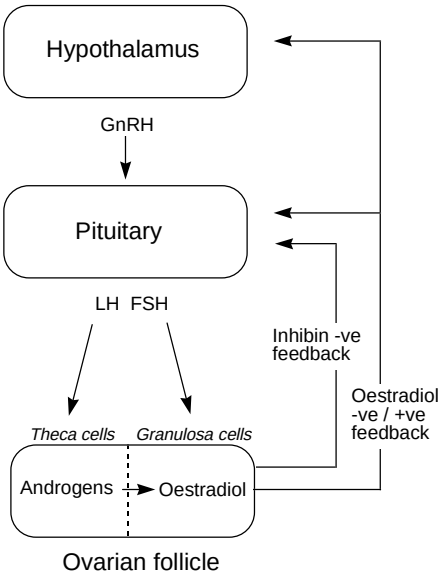

Fig. 4. Overview of the hormonal interrelationships between the hypothalamus, pituitary gland and ovary during the follicular phase of the cycle.

Following ovulation, the corpus luteum develops and progesterone levels rise. Progesterone has a negative effect to slow the frequency of GnRH pulses, and gonadotrophin concentrations decline (see Fig. 5). In women, the corpus luteum also makes inhibin and oestrogen, which serve as potent inhibitors of FSH secretion from the pituitary gland (57, 58). This results in suppressed follicular growth, other than gonadotrophin independent development from the primordial to early preantral stage. The end of the luteal phase is triggered by a process called luteolysis which may involve programmed cell death, or apoptosis (59). In species such as sheep and guinea pig, luteolysis is actively triggered by the release of prostaglandin F2α from the uterine endometrium, and this in turn is stimulated by oxytocin released from the corpus luteum (60, 61). This feedback loop is not found in women, and the mechanisms for inducing luteal regression are not known.

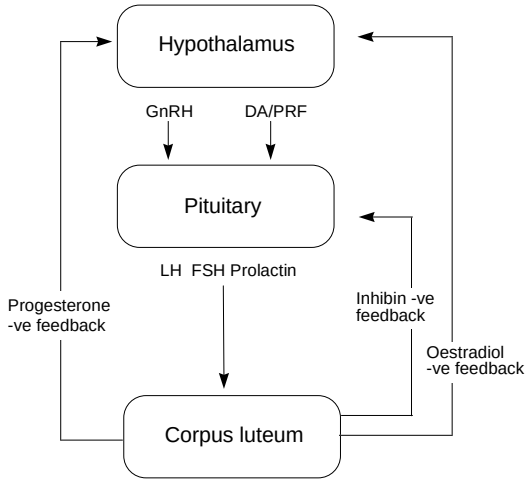

Fig. 5. Overview of the hormonal interrelationships between the hypothalamus, pituitary gland and ovary during the luteal phase of the cycle.

Rat and Mouse

The rat/mouse oestrous cycle is divided into four phases, pro-oestrus, equivalent to the human follicular phase; oestrus; met-oestrus, immediately following oestrus; and di-oestrus which is equivalent to the luteal phase in humans. The main feature of the rat and mouse oestrous cycle is that its length is dependent on whether mating has taken place. In the absence of mating, the luteal phase of the cycle is 2–3 days, but following mating with a vasectomised male, the luteal phase is extended to 11–12 days (62, 63). This extended life-span of the corpus luteum is dependent on the secretion of prolactin from the pituitary gland (64). Mechanical stimulation of the cervix during mating activates an afferent neural pathway to the central nervous system which reduces the amount of dopamine released from the hypothalamus, and prolactin increases (65). Prolactin is an essential part of the complex of hormones required to maintain luteal function, and without it the corpus luteum dies after 2–3 days (62, 64).

Rabbit

The rabbit is an induced ovulator. In the absence of mating, the doe is endocrinologically in a persistent follicular phase. There are waves of follicular growth, which are accompanied by elevated blood oestrogens, and low progestagens. However, mating with a vasectomised buck, causes ovulation some 10–12 hours later, and this is followed by the formation of a corpus luteum and elevated progesterone concentrations (66). The luteal phase lasts 12 days, and if there is no longer a buck present, the doe returns to her persistent follicular state. Like the rat, mechanical stimulation of the cervix activates a neural pathway to the central nervous system, but in this case, results in the generation of an LH surge which triggers ovulation.

ENDOCRINE CONTROL OF THE MALE REPRODUCTIVE SYSTEM

The endocrine mechanisms that regulate testicular activity are similar in many ways to those which regulate ovarian function, and are summarised in Fig. 6. GnRH secreted as pulses from the hypothalamus into the hypophyseal pituitary portal supply stimulates the secretion of LH and FSH. LH acts on Leydig cells to stimulate testosterone synthesis and secretion whereas FSH acts on the Sertoli cell which acts as a nurse cell to support the process of spermatogenesis.

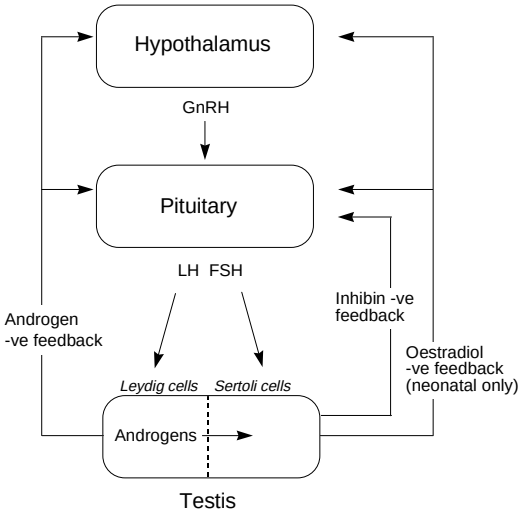

Fig. 6. Overview of the hormonal interrelationships between the hypothalamus, pituitary gland and testis.

Regulation of steroidogenesis

In animals in which the pituitary gland has been removed (hypophysectomy) there is a marked reduction in the rate of androgen synthesis. Androgen synthesis can be restored by exogenous administration of LH (67). The mechanism of action of LH includes the regulation of steroid acute regulatory protein (StAR) which is responsible for the transport of cholesterol from the outer to the inner mitochondrial membrane (40). This makes cholesterol available for conversion to pregnenolone, the first step in the steroid biosynthetic pathway (see Fig. 3). LH is also a trophic hormone for Leydig cells; hypophysectomy causes Leydig cell atrophy, as well as reduced testosterone synthesis and secretion. Prolactin can also stimulate Leydig cell function. Receptors for prolactin are found on Leydig cells, and in rats but not in man, prolactin is known to increase the numbers of LH receptors, thus increasing the capacity for LH to induce steroidogenesis (68).

Regulation of spermatogenesis

Testosterone is essential for the maintenance of normal spermatogenesis. Hypophysectomy in rats halts the process of spermatogenesis but at the same time testosterone production from the Leydig cells is reduced. If these animals are given LH or testosterone at the time of hypophysectomy, Leydig cell function and spermatogenesis is maintained, albeit at a reduced rate (69). In primates this decline in spermatogenesis after experimental hypophysectomy is much more severe. LH or testosterone are insufficient to restore normal spermatogenesis, but if FSH is also given, sperm production returns to normal. The precise role of FSH in the process of spermatogenesis is unclear. FSH acts on Sertoli cells to stimulate the output of many different Sertoli cell proteins including inhibin, androgen binding protein (ABP), and androgen receptor (70, 71). The increase in androgen receptor allows the Sertoli cell to become more responsive to androgen secreted from Leydig cells. The role of FSH in spermatogenesis is also species dependent. Immunoneutralization of FSH using specific antibodies to FSH, results in a marked decline in spermatogenesis in nonhuman primates but has virtually no effect in rats (72, 73). In men with reduced FSH levels, testosterone alone can restore sperm production, although FSH is necessary for this to be complete (74). The difference between species is thought to be due to differences in the effects of FSH on spermatogonial phases of the spermatogenic cycle.

One of the key determinants of sperm output is the number of Sertoli cells in the testis. Each Sertoli cell can only support a finite number of developing sperm cells, so that the number of Sertoli cells sets the upper limit for sperm production. Sertoli cell replication can only occur during a critical window of development in foetal and neonatal life. In rats this window of Sertoli cell replication begins at day 19–

20 of foetal life and finishes at postnatal day 14–15 (75). A similar time span is observed in mice and rabbits. The window for Sertoli cell replication in man is far less clear, but begins during mid-foetal life and extends to at least 1 year post-natally (76). FSH is the single most important determinant of Sertoli cell number (77). Inhibition of FSH secretion during neonatal life in rats will reduce the number of Sertoli cells and this can be reversed by FSH administration (78). By contrast thyroid hormones act by changing the duration of critical window in which FSH can have its effects (79). Thus, neonatal hypothyroidism prolongs the period of Sertoli cell replication giving larger testes with a greater capacity for sperm production. As discussed in previous sections, FSH secretion is subject to negative feedback inhibition by circulating oestrogens. Sertoli cells from neonatal rats secrete oestrogens in response to FSH stimulation (80). These oestrogens maintain the correct concentration of FSH to achieve optimal Sertoli cell replication during the critical window. If exogenous oestrogens are administered during early neonatal life, this results in reduced FSH secretion, and smaller testes with reduced sperm output, in adult life (80). It is therefore also possible for exogenous xenobiotic oestrogens to interfere with this negative feedback pathway, and reduce FSH secretion during the window of Sertoli cell replication. This would lead to a reduction in Sertoli cell numbers and a reduction in the capacity for sperm production in adult life (80–82). This mechanism has been proposed to explain the link between exposure to xenoestrogens and the apparent decline in sperm counts in humans.

CONCLUSIONS

Oestrogens are crucial for the normal development, maintenance and function of the reproductive systems of male and female mammals. On a theoretical basis, xenobiotic oestrogens have the capability of mimicking any of the actions of endogenous oestrogens. Thus, inappropriate exposure to xenobiotic oestrogens could alter the finely balance homeostatic mechanisms which constitute the reproductive endocrine system. The challenge now is to determine if the theory is really translated to reality, by establishing if exposure to xenoestrogens can alter endocrine function in such a way as to have adverse effects on reproductive health.

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