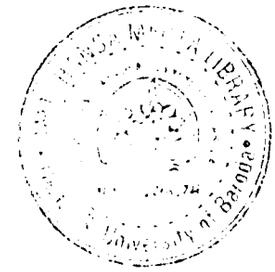

Chapter 1

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Introduction

The endocrine function of the gonads is primarily concerned with perpetuation of the species. The endocrine system is made up of specialized cells, tissues, and organs that create and secrete chemicals in blood called hormones, which then regulate other kinds of cells in the body. Particular hormones only affect particular cells that contain "receptors" for those hormones. A small amount of a hormone attaches to a "receptor" (a protein molecule) and the hormone-receptor pair then initiates a cascade of chemical changes, often with major and far-reaching consequences in remote parts of the body. Thus hormones act as messengers, sending chemical signals that control various functions.

The reproductive process

The survival of any species depends on the integrity of its reproductive system. Reproduction is a complex process requiring interactions among multiple physiological systems. In addition, the two individuals or couple who make up the reproductive unit must also be considered as a target in evaluating reproductive toxicity. The reproductive process is not limited to reproductive organs, but is largely governed by neuroendocrine influence.

Female reproductive physiology

Female reproductive physiology is dependent on the intact functioning of the neuroendocrine system, which involves the hypothalamus, anterior pituitary gland and ovaries. The hypothalamus releases Gonadotropin releasing hormone (GnRH), which acts upon the anterior pituitary gland. In response to GnRH, the anterior pituitary gland secretes Follicle stimulating hormone (FSH) and Luteinizing hormone (LH), which then act upon gonads to secrete gonadal hormones.

Embryogenesis and fetal growth

Following fertilization of the ovum, which occurs between 24 and 48 hours after ovulation. cell division occurs over several days to produce a blastocyst. The blastocyst implants in the lining of the uterus within six to seven days after ovulation. The extraembryonic membranes and the germ cell layers, including the endoderm, mesoderm, and ectoderm. are formed in the second to third week. The period of embryonic development, a critical phase of development, occurs during the eighth to ninth weeks of pregnancy. During this phase the organs are principally formed including the brain, heart. eyes, and limbs. During the fetal period from the eighth or ninth week of pregnancy to gestation, fetal growth is characterized by continued biochemical and physiological maturation.

Function of hormones in brain development and sexual behavior

Hormones not only regulate gametogenesis but also control the dimorphic anatomic. functional and behavioral development of males and females that is essential for sexual reproduction. Differentiation of the male and female brain is determined by the hormonal milieu at a critical developmental stage, a concept supported by the finding of anatomic difference between male and female brains, particularly in the hypothalamus and spinal chord (Gorski, 1983) and by the fact that transplantation of the preoptic regions of the hypothalamus from neonatally androgenised rats into the preoptic region of neonatal females leads to male mating behavior (Arendash and Gorski, 1982).

Men and women pituitary display the spontaneous LH and FSH ultradian rhythms of approximately the same duration, the pacemaker system is allowed in both sexes by the administration of testosterone or progesterone (Spinder et al.. 1989). Although administration of gonadal steroids prenatally may influence certain behaviors. the pattern

of gonadotropin regulation in the higher primates is different than it is in rats and mice. The capacity to develop an increase in LH secretion i.e., the positive feedback response is characteristic of females of all species. In rodents the response of the pituitary and hypothalamus in females to estrogens is prevented if the newborn is exposed to androgens during a critical developmental period (in rats the period extends from birth to fifth postnatal day) (Gorski, 1980). In addition the ability to display adult female patterns of sexual behavior at maturity is lost, even when normal female hormonal balance is restored in adult life.

Behavioral effects of gonadal steroids are mediated through receptors in the brain. Estrogen and progesterone receptors are present in the hypothalamus. After castration, female rats will not mate and genital tract becomes atrophic, both effects being reversible by estrogen treatment. The effect of estrogen on behavior is mediated centrally as shown by the finding that tiny implants of estrogen in the hypothalamus restore normal sexual behavior without reversing the atrophic genital changes. In the hypothalamus progesterone decreases the rate of spontaneous firing and elevates the threshold of excitability to reflex stimulation from the uterine cervix. Progesterone in humans also raises body temperature and is responsible for the postovulatory rise in blood temperature at ovulation.

It is of particular interest in this regard that no exclusive male or female hormones have been identified. All hormones characterized to date are present in both sexes and both sexes have receptor mechanisms that allow response to all hormones. Sexual dimorphism is the result of differences in the amounts of individual hormones and differences in their pattern of secretion, rather than in their presence or absence. It follows that sexual reproduction requires a precise genetic programming that allows for the syntheses of an

appropriate enzyme complement in the ovary or testis that in turn catalyses the formation of appropriate amounts of hormones at the critical stages of life, which are regulated by many factors such as emotions, stress, and environmental inputs. Geoffrey Harris, 1937 showed that electrical stimulation in the hypothalamus of the rabbit could cause ovulation. The portal system between the brain and pituitary was first postulated, that finally became the focus of research and was shown to be connecting path between the hypothalamus and the anterior pituitary gland. Reproductive endocrinology was thus born early in the 20th century and has progressed unbelievably to the present, both as a basic science and in translation to human reproductive health.

Neuroendocrinology

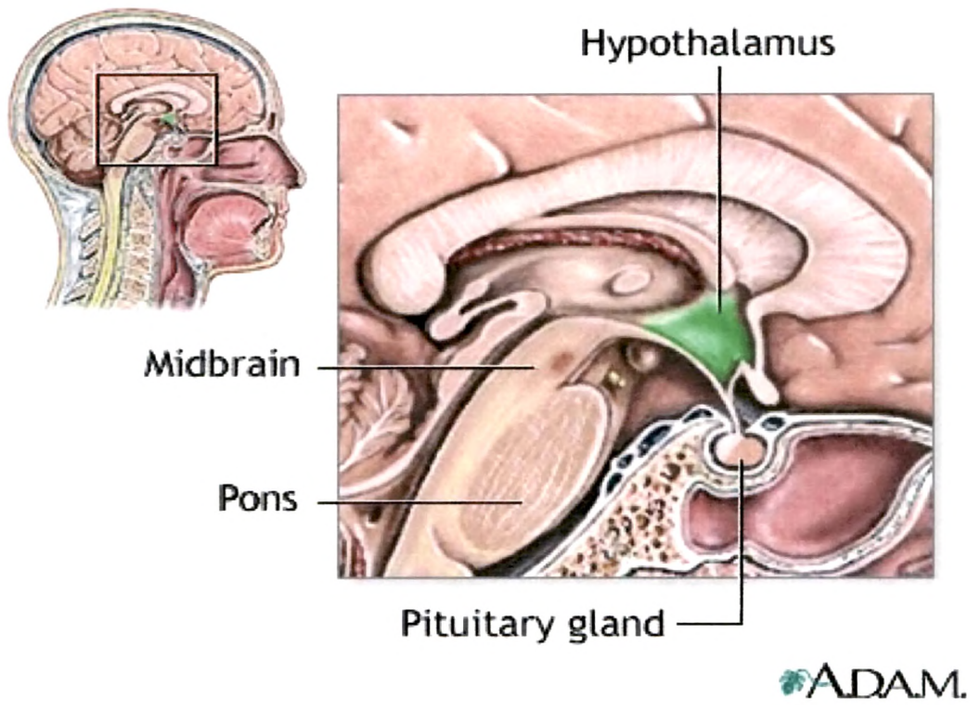
Homeostasis, growth development and reproduction are regulated by the interactions of the endocrine and nervous systems. Almost all endocrine secretions are controlled directly or indirectly by the brain and virtually all hormones influence brain activity. Neurons provide an organized network of point-to-point connections as the basic unit of the nervous system. The basic unit of the endocrine system, the secretory cell, provides the regulatory influence through the circulation. Nerve cells have a secretory function and the capacity to propagate action potentials, and endocrine cells have electric potentials as well as secretory capacity. Neurons in common with endocrine glands activate target cells through chemical mediators that react with specific cell receptors. Any neuronal secretory product from a nerve ending can serve either as a neurotransmitter or a neuromodulator. Neurotransmitters are released into the synaptic cleft and stimulate (or inhibit) postsynaptic neurons. The distinctions between a neurotransmitter and a neuromodulator are not absolute, but neuromodulator tend to have a longer latency before response.

Hypothalamus

The hypothalamus consists of a collection of nuclei and areas located at the base of the brain, ventral to the subthalamus. Along most of it's length, the lateral border is formed by the fibers of the internal capsule. Posteriorly, the posterior hypothalamic division is continuous with the midbrain. The most anterior division of the hypothalamus, the preoptic region, is bounded by the basal forebrain. The arcuate region is next to the posterior region, and the fourth division, the anterior hypothalamus, is between the arcuate and preoptic regions. The hypothalamus is connected anatomically to both the forebrain and brain stem (Fig. 1).

The median eminence of the hypothalamus is the site at which anterior pituitary regulating hypothalamic neurons release their secretions into the capillaries of the primary plexus of the hypophyseal portal system. The median eminence has three components; **neural**, consisting of nerve terminals and neurons in passage; **vascular**, consisting of the primary capillary plexus and the portal veins; and **epithelial,** consisting of the pars tuberalis of the anterior pituitary gland. Nerve endings in the median eminence are terminals of the tuberhypophyseal neurons, which arise chiefly in the mediobasal hypothalamus and include fibers from the supraoptico- and paraventricular magnicellular neurons. Two types of tuberhypophyseal neurons project into the median eminence, peptide secreting (peptidergic) (e.g.: GnRH, GHRH, TRH, LHRH and Somatostatin) and bioaminergic the most important of which are dopaminergic. Some peptides in nerve endings are not released into the hypophyseal-portal circulation but instead function to regulate the secretion of other nerve terminals. The primary direction of blood flow is from the hypothalamus to the pituitary and interruption of this connection leads to a decline in

Fig 1: Hypothalamus- an overview



gonadotropin levels and eventually to atrophy of the ovaries with failure of hormone secretion. Retrograde flow also occurs in the portal vessels providing a short feedback loop from pituitary to hypothalamus.

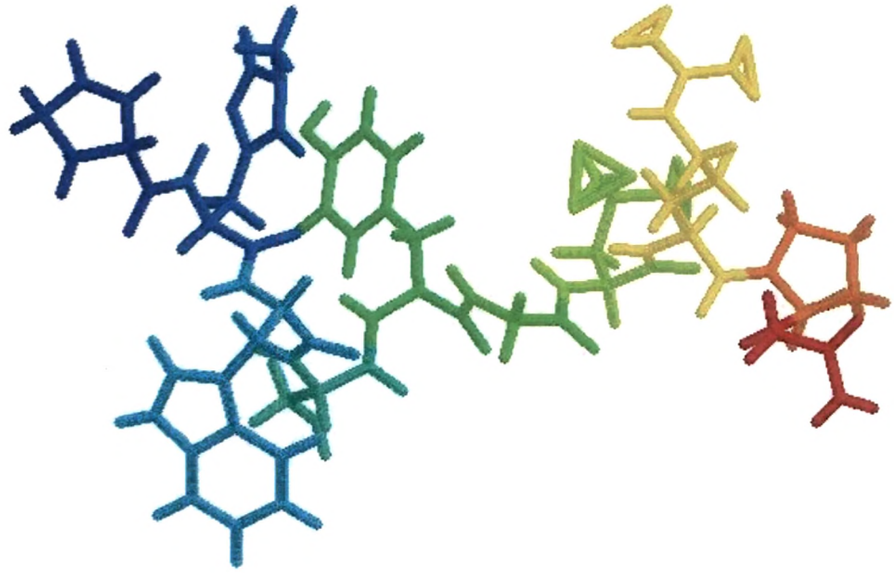
The hypothalamus plays an important role in the hormonal regulation of female reproductive function. Understanding of the hormonal control of reproduction has progressed from the identification of ovarian steroids and pituitary gonadotropin to the discovery of hypothalamic releasing factors and the identification of a host of ovarian hormones and growth factors that modulate gonadotropin secretion and intraovarian regulation.

GnRH (also called LHRH)

GnRH, a decapeptide, like other neuropeptides is synthesised at the arcuate nucleus in hypothalamus as part of a large hormone that is cleaved enzymatically and further modified within secretory granules. Most of the GnRH in mammalian brain is in the hypothalamus and related neural structures, but it is also found in the limbic system, including the hippocampus, cingulate cortex and olfactory bulb, structures that are responsible for emotional expression. GnRH is expressed in the placenta and stimulates placental secretion of chorionic gonadotropin (Belisle et al., 1984).

Seven different forms of GnRH have been demonstrated in different species. All are 10 aminoacid containing peptides (decapeptide) and all have at least 50% homology to mammalian GnRH (Sherwood et al., 1993). All forms have identical Pyro-Glu amino acid at N-terminal and Gly amide at carboxy terminal. As with other regulatory peptides the nucleotide sequences encoding the functional peptide are highly homologous, but the intervening sequences show wide divergence. Response elements for atleast three nuclear

Fig 2: GnRH peptide structure



regulatory steroids –nicotinic acid, estrogen and thyroid hormone are found in this gene (Marshall and kelch, 1986). The preprohormone on cleavage yield 23 amino acid signal peptide followed by a decapeptide (GnRH) and 56 amino acid peptide designated as GAP (Gonadotropin associated peptide) (Fig. 2). It is variable among species and unlikely to be hormonally important. Little evidence suggests prolactin releasing activity of GAP.

Functions of GnRH

Some workers believe that two different hypothalamic factors regulate the secretion of gonadotropins. one stimulating LH secretion and the other stimulating the release of FSH. Others believe that inhibin: a peptide secretion of the gonads is shown to selectively inhibit FSH secretion. The rate at which GnRH pulses are administered alters the pattern of LH and FSH, slower frequencies increases FSH relative to LH and constant infusions suppresses secretion of both LH and FSH. The administration of antisera against GnRH inhibits the secretion of both LH and FSH. For this reasons. the view that there is only one hormone that releases gonadotropins is widely held.

A single intravenous injection of GnRH causes a dose related increase of LH and FSH in all vertebrate species. The response is influenced by previous GnRH exposure. the gonadal steroid milieu: gender. the stage of sexual maturation and the timing of administration of the hormone. GnRH has been also implicated in sexual drive in rats, but not in monkeys and probably not in humans. Other potential sites of action correspond to the distribution of GnRH receptors and include human ovary. prostate. testis and lymphocytes.

Regulation of GnRH secretion

The area of the brain responsible for the GnRH pulse generator is in the arcuate nucleus of the medial basal hypothalamus. The rhythmic nature of GnRH pulse generator is regulated

by the hormonal milieu. Testosterone slows the rate of discharge which is the main mechanism by which testosterone inhibits gonadotropin release. Progesterone also slows the pacemaker whereas estrogen has no effect on the pacemaker.

The half life of GnRH is short (2 to 4 min) and its metabolic clearance rate average 800L/m2 body surface area/d. The fact that LH and FSH secreted in short pulsatile bursts led to the assumption that GnRH release is also pulsatile. GnRH is secreted in a pulsatile fashion at intervals of 70 to 90 min.

The mechanism by which preovulatory secretion of estrogen and progesterone triggers the midcycle surge of gonadotropin is not well understood. In all species, gonadotropin responsiveness to GnRH increases at midcycle either because of sensitisation of pituitary gonadotrophs by estrogens or because of stimulation of GnRH secretion, or both, which in turn upregulates GnRH receptors. GnRH secretion is enhanced at midcycle in rodents and rhesus monkey (Pau et al., 1993). Since GnRH neurons do not have estrogen receptors, enhanced GnRH secretion is not caused by direct action of estrogens. Rather estrogens act through cells that have estrogen receptors such as ascending noradrenergic fibers from the locus coëruleus in the brain stem and neurokinergic fibers that arise in the hypothalamus (Rance and Uswandi, 1996).

Neural control of GnRH secretion is mediated by signals from four classes of neurotransmitters-bioamines, neuropeptides, excitatory amino acids and gaseous neurotransmitters. Excitatory factors include norepinephrine acting through β1 receptors. neuropeptide Y, galanin, NT, substance P and other tachykinins. glutamic acid. NO. transforming growth factor α and prostaglandin E2. Under appropriate conditions any of these factors can release GnRH, pharmacologic blockade of any single factor can block or

reduce the GnRH ovulationary surge. Epinephrine and norepinephrine increase GnRH release, whereas dopamine and serotonin and endogenous opiod peptides are inhibitory (Shivers et al., 1983). Central opiodergic neurons tonically suppress GnRH secretion except during the ovulationary surge when they are not inhibited. GnRH secretion is also reduced by Corticotropin releasing hormone (CRH), vasopressin and inflammatory cytokines. Other hormones in particular gut related peptide hormones also modulate GnRH release.

GnRH receptor subtypes

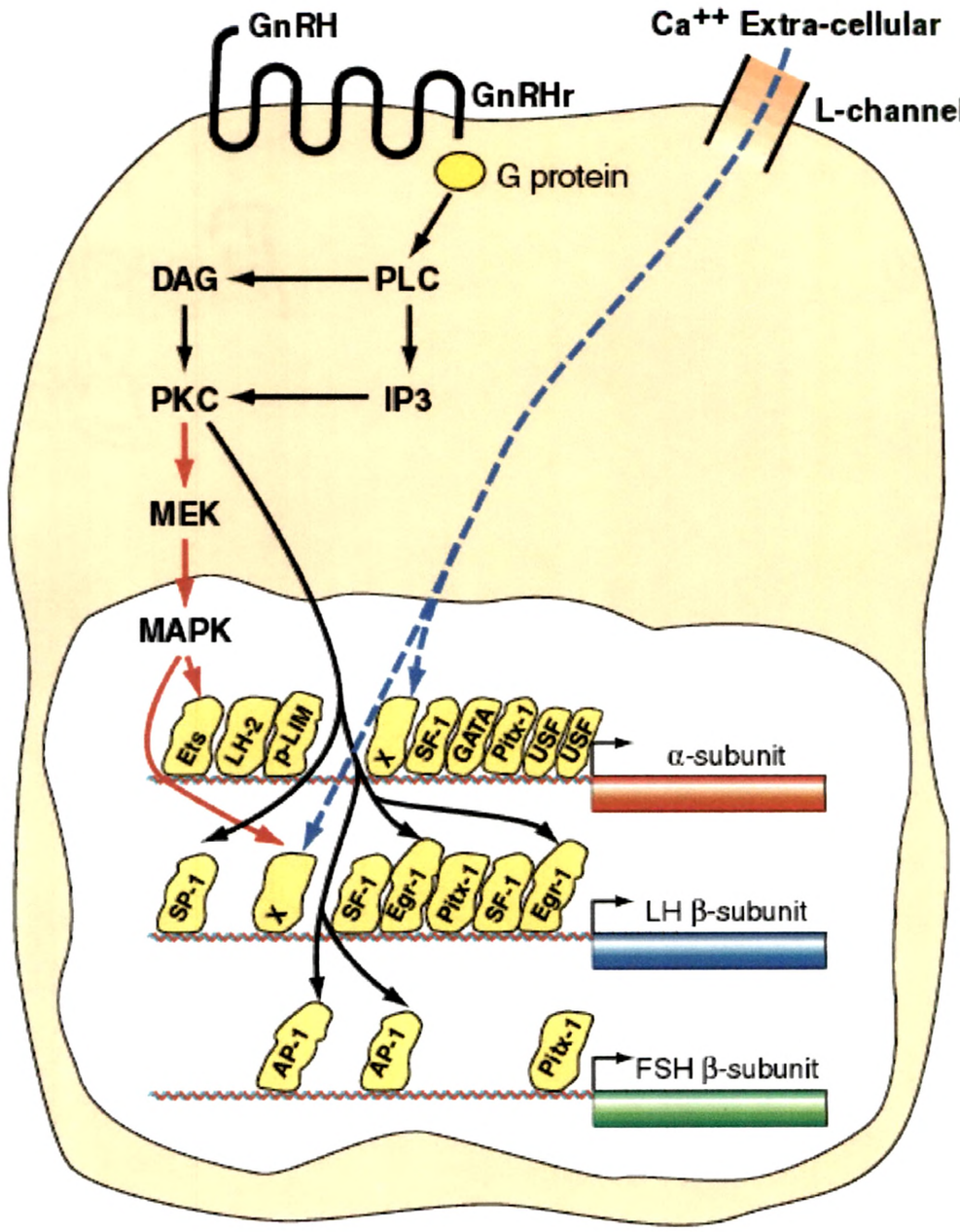
Mammalian gonadotropin-releasing hormone (GnRH I) is a hypothalamic decapeptide that governs gonadotropin secretion through interaction with its seven transmembrane (7TM), G protein-coupled receptor (GPCR) expressed by anterior pituitary cells. A second decapeptide, GnRH II, originally discovered in the chicken hypothalamus was recently reported to be expressed in the mammalian hypothalamus as well (Neill, 2002). A search of the recently sequenced human genome identified a 7TM/GPCR on chromosome 1 that exhibited a higher identity with non-mammalian vertebrate GnRH II receptors (55%) than with the human GnRH I receptor (39%). Molecular cloning and nucleotide sequencing of this putative GnRH II receptor cDNA from monkey pituitary gland revealed a 379 amino acid receptor that, unlike the GnRH I receptor, possessed a C-terminal tail. Heterologous expression and functional testing of the receptor in COS-1 cells confirmed its identity as a GnRH II receptor: measurement of ^{3}H-inositol phosphate accumulation revealed EC(50)s for GnRH II of 0.86 nM and for GnRH I of 337 nM (Neil, 2002). Ubiquitous tissue expression of GnRH II receptor mRNA was observed using a human tissue RNA expression array and a ^{32}P-labeled antisense riboprobe representing the 7TM region of

human GnRH II receptor cDNA. As predicted by the presence of its C-terminal tail, the GnRH II receptor was desensitized by GnRH II treatment whereas GnRH I receptor was not desensitized by GnRH I. Pharmacological analysis of the GnRH II receptor revealed that GnRH I 'superagonists' are more potent than GnRH I but less potent than GnRH II. Estrogens sensitize the pituitary to GnRH and thus increase and androgens decrease the number of GnRH receptors. GnRH receptors are also present in the ovary and testis of rat and in human ovary. Although GnRH stimulates the release of steroid hormones from isolated rat ovaries it is doubtful that circulating GnRH has a role in gonadal functions because its concentration in general circulation is very low.

GnRH signalling

GnRH action is initiated by binding to specific cell surface receptors, leading to activation of a specific G protein (Gq/11), stimulation of multiple phospholipase activities in the plasma membrane, differential modulation of inositol 1,4,5 triphosphate and diacyl glycerol signals, and cytoplasmic calcium response. Sustained stimulation of G-protein coupled receptors (GPCRs) typically causes receptor desensitization that is mediated by phosphorylation, often within the C-terminal tail of the receptor. The consequent binding of β-arrestin not only prevents the receptor from activating its G-protein (causing desensitisation) but can also target it for internalization via clathrin-coated vesicles and can mediate signalling to proteins regulating endocytosis and mitogen-activated protein kinase (MAPK) cascades (Fig. 3). The type I GnRH-receptors (GnRH-Rs) do not undergo agonist-induced phosphorylation or bind β-arrestin. In contrast, the types II GnRH-Rs show rapid desensitisation and internalization with concomitant receptor phosphorylation (within the C-terminal tails) and/or binding of β-arrestin. The binding to β-arrestin may

Fig 3: GnRH signal transduction



also be important for association with dynamin, a GTPase that controls cleavage of endosomes from the plasma membrane. Using recombinant adenovirus to express GnRH-R, it was found that blockade of dynamin-dependent endocytosis inhibits internalisation of type II (Xenopus) GnRH-Rs but not type I (human) GnRH-Rs, revealing the existence of functionally distinct routes through which these receptors are internalised (McArdle et al., 2002). Although type I GnRH-R do not rapidly desensitize, sustained activation of GnRH receptors does cause desensitisation of gonadotropin secretion, an effect which must therefore involve adaptive responses distal to the receptor. One such response is shown to be GnRH-induced down regulation of inositol 1, 4, 5 triphosphate receptors that apparently underlie desensitisation of Ca^{2+} mobilization in a gonadotroph-derived cell line (Forrest-Owen et al., 1999). Although activation of other GPCRs can down-regulate inositol 1, 4, 5 triphosphate receptors, the effect of GnRH is atypically rapid and pronounced, presumably because of the receptor's atypical resistance to desensitisation.

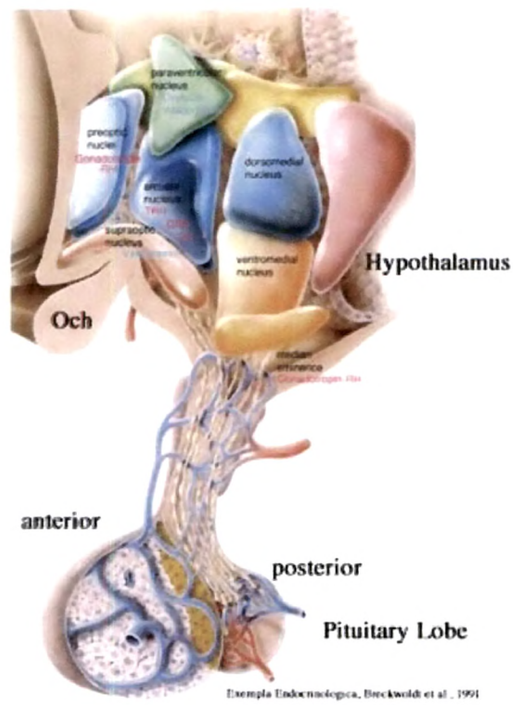
GnRH-Rs are also expressed in several extra-pituitary sites and these may mediate direct inhibition of proliferation of hormone-dependent cancer cells (Grundker et al., 2002). Infection with type I GnRH-R expressing adenovirus facilitated expression of high affinity, PLC-coupled GnRH-R in mammary and prostate cancer cells and these mediated pronounced antiproliferative effects of receptor agonists. No such effect was seen in cells transfected with a type II GnRH-R, implying that it is mediated most efficiently by a non-desensitizing receptor. Thus it appears that the GnRH-Rs have undergone a period of rapidly accelerated molecular evolution that is of functional relevance to GnRH-R signalling in pituitary and extra-pituitary sites.

The anterior pituitary lobe (Adenohypophysis)

The adenohypophysis is an endocrine gland. In most vertebrates the pituitary is divided into three clearly defined lobes (Fig. 4). The anterior lobe (also called adenohypophysis, pars distalis or pars glandularis), the neural lobe (also called the posterior pituitary or the infundibular process) and the intermediate lobe (also called the pars intermedia). Each area of the pituitary plays a unique role in the endocrine system. The *adenohypophysis* is a heavily vascularized region consisting of numerous capillaries. It is arranged in irregular chords or clusters composed of cells known as *chromophobes* and *chromophils*. Chromophobes, also referred to as folliculo-stellate cells. Chromophils can be further categorized into *acidophils* and *basophils*. Acidophils stain using acidic dyes, and basophils stain using basic dyes. The acidophils stain orange and the basophils stain red-blue. This region of the pituitary produces several hormones such as LH, FSH, TSH, GH, Prolactin and POMC. The pars intermedia produce the hormone MSH. The pars nervosa is the neural component of the pituitary gland. It contains the terminal axons of neurosecretory neurons with cell bodies in the hypothalamus. This region stores and releases the hormones oxytocin and ADH produced by the neurosecretory neurons whose cell bodies are located in the hypothalamus.

The release of these adenohypophysial hormones from epithelial cells in the gland is regulated by hypothalamic hormones (hypophysiotropic hormones). also termed releasing hormones and release-inhibiting hormones. They are secreted from small neurons in the hypothalamus and are carried by the circulation from the median eminence (as described earlier) into the adenohypophysis, diffuse from the secondary capillary bed to reach the secretory epithelial cells of the adenohypophysis, may facilitate or inhibit the synthesis and

Fig 4: Pituitary- an overview



release of the pituitary hormones (tropic hormones). Upon their release, the tropic hormones enter the venous sinus of the gland, and then via the general circulation to remote target sites (peripheral glands and tissues). Altogether, the hypothalamic-anterior pituitary axis is considered an important link between the brain and the endocrine system (Stanfield, 1960).

Gonadotropes

Gonadotropes are ovoid, medium sized or smaller and widely distributed throughout the pars distalis of the pituitary, their prevalence in the human gland is about 20%. The morphologic features and immunoreactivity of gonadotropes vary with different hormonal states. The gonadotrope elaborates both LH, and FSH during the menstrual cycle. The secretion of the hormones varies and their concentrations in the gonadotrope change. The cells have a large interface with capillary basement membranes and are often close to lactotropes, raising the possibility of a paracrine interaction between the two. Gonadotropes have spherical conspicuously euchromatic nucleus, and low-density cytoplasm that contains well developed mildly dilated RER and a prominent golgi apparatus. In women the secretory granules tend to be larger (200 to 600 nm) but no specific histologic changes occur with physiological hormonal variations. In men small (<200 nm) secretory granules are usually more numerous.

Gonadotropins

Gonadotropins belong to glycoprotein hormone family made up of 2 subunits α and β. α subunit is common to both LH and FSH. α subunit gene is located on chromosome 6 having 4 exons and 3 introns. Prohormone is synthesized as 116 amino acids and mature molecule having 92 amino acids with two N-terminal oligosachharide units.

The human LHβ subunit gene is located on chromosome 19q 13.32 and is close to the hCG β genes. Each of the glycoprotein β subunit is coded by a single gene except hCG β. There are atleast seven hCG β genes and pseudogenes, and only primates and horses are known to have these genes. The LH and hCG β genes are approximately 1.5 kb size and consists of three exons and two introns. The LH β gene encodes a prohormone with a 24-aminoacid leader sequence a mature peptide of 121 amino acids. The hCG β protein differs from other β subunits in having a 24 aminoacid –COOH terminal extensions, and a longer 5' untranslated portion. The 5'-flanking region of the LH β gene contains a putative estrogen response element.

The human FSH β gene is located on chromosome 11p13 and contains three exons and two introns. The nucleotide and aminoacid sequences of the coding regions of the rat and human FSH β subunits show 79 and 80% homology, respectively. In contrast to a single mRNA in rat and cow, the human FSH β subunit gene is transcribes into four mRNA, as the result of alternate splicing of exon 1 and two polyadenylation sites.

The half time of immunoreactive FSH is greater than that of LH. Similarly metabolic clearance of FSH is less than that of LH, being 6 to 14 ml/min in women and 4 to 12 ml/min in men. Gonadal function does not affect the metabolic clearance rate. The liver and kidney degrade circulating LH and FSH; small amounts of intact LH and FSH are excreted in the urine (3 to 5% for FSH). The content of gonadotropins is low in the pituitary of prepubertal children. In men and in menstruating women the pituitary contains approximately 700 IU of FSH. After menopause the content of pituitary LH rises to approximately 1700 IU but there is no change in FSH content. The prolonged bioactive half-lives of LH and FSH are due to the oligonucleotide content. Asialoglycoproteins

(protein stripped of sialic acid) are cleared rapidly by liver. The range of both serum LH and FSH in normally cycling adult woman is 5 to 20 IU/L. However, on or about the day of ovulation, levels of FSH and LH may be two to three times normal values. A serum level of FSH higher than 40 IU/L is diagnostic of ovarian failure.

Gonadal maturation is regulated by gonadotropins. These hormones are also responsible for the timing and control of pubertal development and sexual maturation. Before puberty the release of FSH is greater than that of LH, the relationship is reversed at puberty. The differential release of LH or FSH is not well understood, but may reflect the effects of inhibin. The major restraint on prepubertal GnRH secretion originates in central nervous system.

Both LH and FSH are required for estrogen syntheses, and the amount of estrogen produced depends on the relative exposure to each gonadotropins. FSH is required for follicular maturation and growth and FSH receptors are present exclusively on the granulosa cells. An enhanced secretion of estradiol causes further proliferation of granulosa cells and follicular growth and an increase in the number of estradiol receptors including estradiol receptors in granulosa cells. In a mature follicle, FSH content in concert with estradiol causes an increase in LH receptors on granulosa cells. LH acts in granulosa cells through these receptors to augment progesterone secretion which then increase FSH secretion at midcycle. After ovulation. the number of LH receptors in the lutein cells increases and the number of FSH receptors and degree of FSH response decrease.

Regulation of pituitary hormone secretion

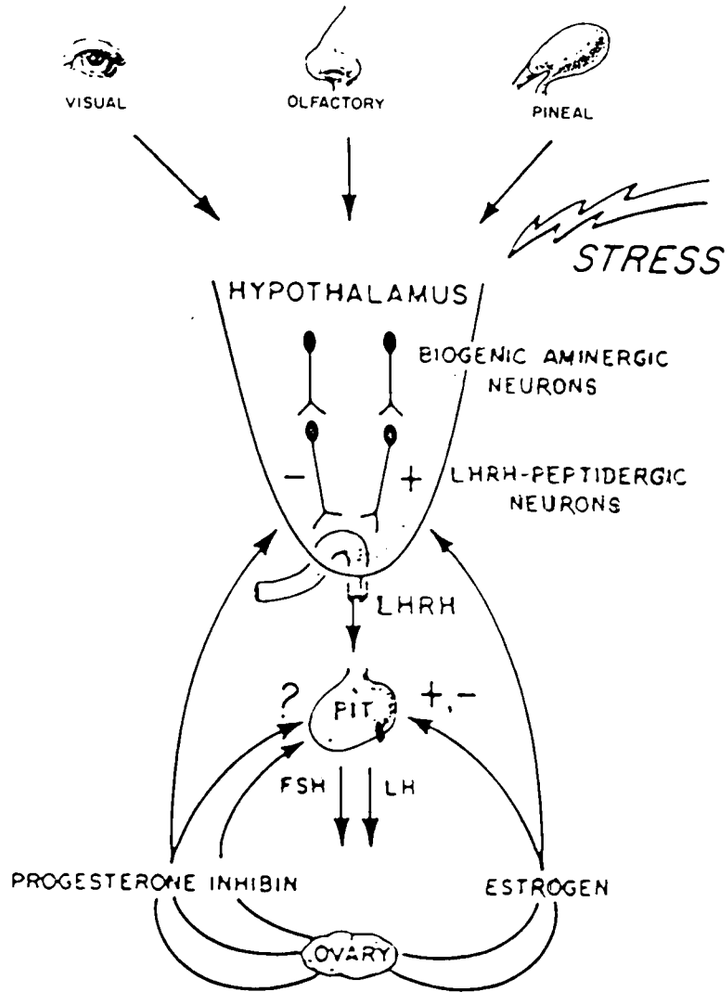
GnRH is essential for the gonadotropin secretion. and the timing of GnRH pulses is crucial in the regulation of LH and FSH secretion. The frequency and amplitude of GnRH pulse

are important in regulating LH and FSH secretion differently. The pulsatile secretion of both LH and FSH is mediated with one GnRH pulse per hour, more frequent pulses initially increase the frequency of LH pulses and mean LH concentration. In contrast, when the GnRH pulse frequency is decreased to once every 3 hour, FSH secretion is preferentially stimulated.

In vitro studies have confirmed that the administration of GnRH in a pulsatile fashion increases transcription of α subunit and LH β by rat anterior pituitary fragments. Continuous GnRH exposure stimulated α subunit mRNA syntheses threefold whereas pulsatile GnRH administration increased the transcription of LH β mRNA and FSH β mRNA and α subunit mRNA. Under certain conditions by reverse flow hypophysial hormones themselves are feedback signals that can affect the hypothalamus. Such signaling is termed short-loop feedback, because it does not involve the general circulation. In another form of regulation, ultra-short-loop feedback, the presence of releasing hormones in the median eminence provides negative feedback paracrine regulation by inhibiting their own release from the hypothalamus (Fig. 5).

Steroid hormones have a profound influence on the secretion of gonadotropins. FSH and LH. These effects can occur as a result of steroid hormones modifying the secretion of GnRH from hypothalamus (direct long-loop feed back effect), or a direct effect of steroid hormones on gonadotropin secreting cells in the anterior pituitary gland (direct short- loop feed back effect). They act by binding to gonadotrope nuclear receptors that affect transcription of various genes by binding to appropriate response elements on DNA. In general the short term (24 h) effects of estradiol are stimulatory for synthesis and secretion of LH by increased sensitivity to GnRH, via increased synthesis of GnRH receptors. In

Fig 5: Regulation of LH and FSH



contrast, the effect of progesterone is inhibitory to syntheses and secretion of LH. Steroid effects on gonadotropin synthesis and secretion may be mediated via: 1) Direct interaction of a steroid hormone receptor with a response element on a gonadotropin gene, as appears to occur with the effect of estradiol on LHβ; 2) Indirectly via the steroid hormone altering production of another protein which in turn affects the gonadotropins, as appears to occur with estradiol inhibiting the expression of the activinβ gene that leads to a decrease in synthesis of FSH (Baratta et al., 2001) and 3) By altering hormone production of another gene that influences activity of gonadotropes as occurs when progesterone suppresses secretion of GnRH from hypothalamus, in turn leading to decreased expression of GnRH receptor (Turzillo et al., 1995). Thus LH pulse frequency is decreased during the luteal phase of the menstrual cycle. Androgens decrease levels of α and LH β mRNAs by suppressing GnRH secretion, but have no effect on FSH β mRNA. Testosterone inhibits human alpha gene expression.

Gonadal peptides have important role in the secretion of gonadotropins and possibly other pituitary hormones.

Inhibin: Inhibin plays an important role in the regulation of FSH secretion. Gonadotropin. growth factor and gonadal steroids regulate gonadal secretion of inhibin. Inhibin is a member of a longer family of glycoprotein hormones. Inhibin, type α βA and α βB inhibit FSH secretion. Inhibin is synthesised by sertoli cells of the testis. granulosa cells of the ovary. the placenta. pituitary gonadotropes and the brain. Thus inhibin may regulate GnRH and gonadotropin secretion not only as a hormone but also by local production as an autocrine or paracrine factor. FSH stimulates ovarian inhibin secretion. Inhibin selectively reduces levels of FSH β subunit mRNA. Inhibin B is secreted by small ovarian follicles

whilst, inhibin A is secreted by the growing dominant follicle and the corpus luteum. There is evidence for inhibin B as a marker of ovarian reserve in reproductive aging. Inhibin B is also the only molecular form of inhibin in male circulation. Inhibin A is the major molecular form of inhibin in maternal circulation. **Activin:** There are three molecular forms of activins; activin A (βA-βA dimer), activin AB (βA-βB dimer) and activin B (βB-βB dimer). Activin stimulates pituitary FSH secretion. Activin selectively increases levels of FSH β subunit mRNA. Activin A is in maternal circulation throughout pregnancy. Feto-placental unit is the major source of these proteins. Maternal circulating levels of these proteins are higher in abnormal pregnancies such as pre-eclampsia.

Follistatin, a single chain glycosylated ovarian glycoprotein is not a member of the inhibin family selectively decreases levels of FSH β mRNA and inhibits FSH secretion in vitro, as does inhibin. They are monomeric binding proteins of activins and inhibins. Binding of follistatin to activin completely negates activin action. Thus while inhibins (whether in free or bound state) retain their biologic activity, activins are only active when not bound to follistatins. In contrast to the dynamic changes in circulating inhibin levels, total follistatin levels remain constant during different phases of the human menstrual cycle. The role of peripheral follistatin is believed to be that of restricting the action of activin to its site of production. This is also supported by the finding that very little detectable free activin A and B exist in the circulation.

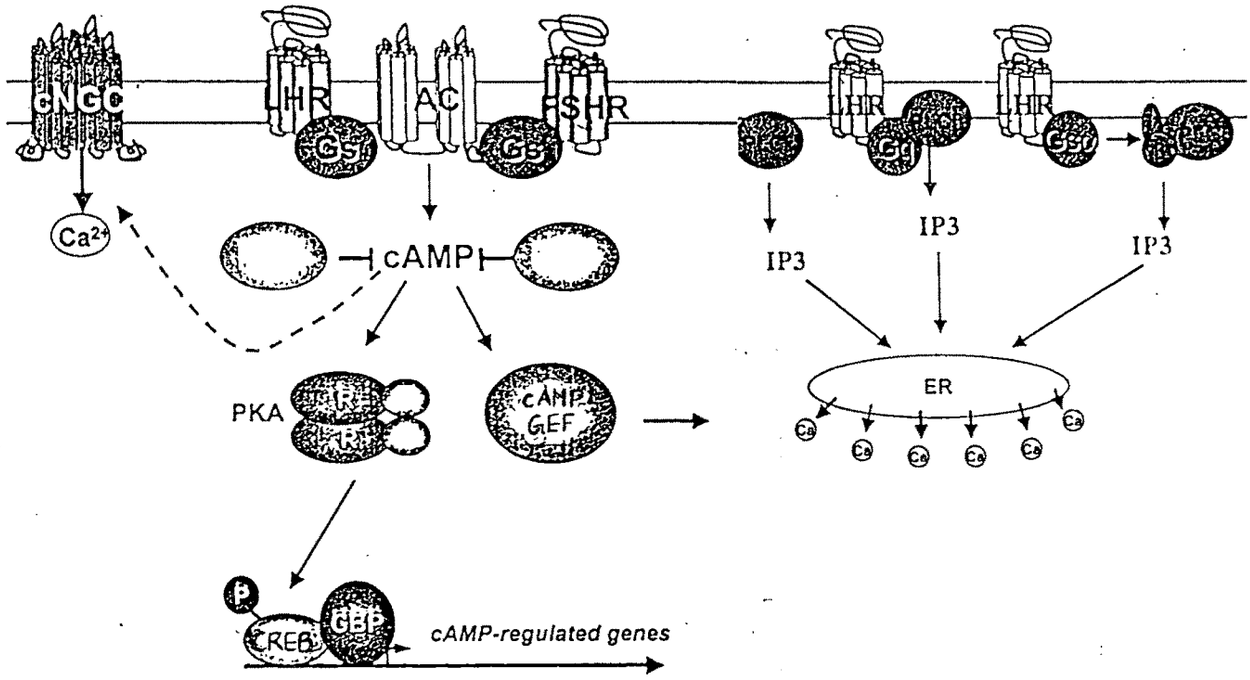
Estrous cycle: During estrous cycles regulation of LH and FSH secretion is dissociated. Steady state mRNA levels and transcription of RNA encoding α, LH β and FSH β subunits have not been measured in primate pituitaries during the menstrual cycle, but changes in both the steady state levels and transcription rates occur during the rat estrous cycle. The

proestrous LH surge at 7 PM is preceded by an increase in LH β mRNA levels at 6 PM. A rapid increase in the FSH β mRNA level occurs at 8 PM immediately after the LH surge and α subunit mRNA levels do not change at the time of the preovulationary LH surge. A modest increase occurs in both α and LH β levels at 8 PM in diestrous. In addition FSH mRNA levels increase from 11 PM in estrous until 5 PM in diestrous. Similarly transcription rates for LH β mRNA are twofold higher on the afternoon of the proestrous when compared with metaestrous and FSH β mRNA transcription is increased at proestrous and continued to increase until the morning of metaestrous.

Mechanism of gonadotropin signal transduction

Both LH and FSH receptors belong to the large superfamily of G-protein coupled receptors (GPCRs) with the distinctive structural characteristics of a large leucine rich extracellular domain. The leucine rich repeats are thought to be important for glycoprotein hormone binding. Because of this unique feature, the TSH, LH and FSH receptor (FSHR) are segregated into a subfamily of the so called leucine rich repeat-containing G-protein coupled receptors (LGRs), which includes the more distantly related LGR4-8 (Hsu et al., 2000). Given their seven transmembrane topology, both LH receptors (LHRs) and FSHRs signal. by coupling to heterotrimeric G proteins. A coupling to Gs, and therefore to adenyl cyclase. has been directly observed in membranes from porcine follicles. In the case of granulosa cells and follicular development and maturation, one could envisage that the FSHR predominantly activates the cAMP-signaling pathway, whereas the LH receptor utilizes additional second messengers i.e., Ca++ and phospholipids. and the LH divergent effects are the result of the activation of the two pathways (Richards et al., 1995) (Fig. 6).

Fig 6: Gonadotropin signal transduction



cAMP cascade activates three classes of the newly discovered class of cAMP activated guanine nucleotide exchange factors (cAMP-GEF or EPAC), cyclic nucleotide gated channels (CNGC) and cAMP dependent protein kinases (PKAs), the latter being the best characterized effectors in granulosa cells. It is established that cAMP binds to the regulatory subunit of PKA, which then either phosphorylates cytoplasmic substrates or is translocated to the nucleus phosphorylate transacting factors via the cAMP regulatory element binding protein (CREB) and the CREB binding protein-CBP/p300, a transcription co activator. Activation of cAMP signaling also causes activation of long splicing variants of PDE4s expressed in granulosa cells and theca cells, which may be responsible for the transient nature of the cAMP signal induced by the LH surge. FSH promotes massive growth of granulosa cells in the preantral follicle by regulation or induction of genes involved in cell cycle control and stimulates androgen aromatization via activation of the cAMP-signaling cascade.

Several reports have indicated that the cAMP pathway in granulosa cells is connected to other signaling pathways. Thus FSH readily stimulates cAMP mediated ERK phosphorylation and the p38 mitogen activated protein kinase (p38MAPK). An additional kinase cascade distinct from the RAF-MEK-ERK, is PI-3 kinase-signaling pathway. An association between LH receptor activation and an increase in intracellular Ca++ in granulosa cells has also been observed (Veldhuis and Klase, 1982; Veldhuis, 1987). However, differences were observed between the behavior of the LH receptors and FSHRs coupling to IP_3 formation, with LHR being more effective than FSHR. LHR coupling to PI turnover may indicate that during the terminal differentiation of granulosa cells, occupancy of this receptor activates two parallel signaling cascades. Indeed, it has been proposed that

PLC and protein kinase C (PKC) activation are required for granulosa cell luteinisation. Regulation of intracellular calcium concentration in granulosa cells by gonadotropins may not be directly dependent on the coupling of the receptors to a G protein that activates PLC. For instance, it is possible that βγ subunits released from the activation of the heterotrimeric Gs may interact and activate PLCβ. In addition, accumulation of Ca^{++} in granulosa cells may be distal to cAMP accumulation and dependent on the expression of other PLC isoenzymes regulated by other effectors.

Ovarian steroidogenesis

The ovary is responsible for the control of reproduction through its principal products, oocytes, steroid and protein hormones. The ovary itself has three functional subunits: the follicle, the oocyte and the corpus luteum. During the normal menstrual cycle, these components, under the influence of FSH and LH, function in concert to produce a viable ovum for fertilization and a suitable environment for implantation and subsequent gestation.

During the preovulatory period of the menstrual cycle, follicle recruitment and development occur under the influence of FSH and LH. The latter stimulates the production of androgens by theca cells, whereas the former stimulates the aromatization of androgens into estrogens by the granulosa cells and the production of inhibin, which acts at the anterior pituitary to decrease the release of FSH. This prevents excess stimulation of follicular development and allows continuing development of the dominant follicle—the follicle destined to ovulate. Estrogen production increases, stimulating both the LH surge (resulting in ovulation) and the cellular and secretory changes in the vagina, cervix, uterus and oviduct that enhance spermatozoa viability and transport. In the postovulatory phase,

theca and granulosa cells remaining in the follicular cavity of the ovulated ovum form the corpus luteum and secrete progesterone. This hormone stimulates the uterus to provide a proper environment for implantation of the embryo if fertilization occurs.

All the steroids of the ovary as well as those produced by the fetus, adrenal and placenta are derived from cholesterol. Cholesterol can be obtained from three sources: 1. Preformed cholesterol circulating in the blood, 2. Cholesterol synthesized denovo (acetyl coenzyme A) and 3. Cholesterol liberated from cholesterol esters. The cholesterol used by the human ovary for steroidogenesis is derived primarily from the uptake of plasma LDL cholesterol. Cholesterol is then transported to the inner mitochondrial membrane by cyclic AMP activated steroidogenic acute regulatory (stAR) protein, a 30 kd mitochondrial protein that is believed to be the key mediator of the acute induction of steroidogenesis. The conversion of cholesterol to pregnanalone is the rate-limiting step in ovarian steroidogenesis; it is catalysed by a cholesterol side chain cleavage enzyme consisting of Cytochrome 450 side chain cleavage enzyme (CYP 450), adrenodexin and flavoprotein.

The principal steroid producing cells of the ovary –namely the granulosa, theca and corpus luteum cells- possess the complete enzymatic complement required for steroid hormone formation. The main pathway of steroid syntheses in the human corpus luteum is the Δ4 pathway, which involves conversion of pregnanalone to progesterone. In the ovarian follicle the Δ5 pathway, is preferred for the formation of androgens and estrogens. The factors that determine which steroid is secreted by each cell type include the levels of gonadotropins (as discussed earlier) and gonadotropin receptors, the expression of steroidogenic enzyme and the availability of LDL receptor.

The rate of steroid production during the menstrual cycle is a function of the content of four key enzymes, CYP11A1, 3β HSD, 17 α hydroxylase (CYP17) and aromatase (CYP19). The enzymes catalyze respectively the conversion of cholesterol to pregnanalone, pregnanalone to progesterone, progesterone to androgens and androgens to estrogens. LH regulates the conversion of cholesterol to pregnanalone and FSH regulates the conversion of androgens to estrogens.

The regulation of steroid hormone synthesis is further complimented by substrate availability. Granulosa cells of the follicle are avascular and extremely low levels of LDL are found in the follicular fluid that bathes granulosa cells. As a result, the granulosa cells have limited ability to form progesterone. The androgens produced in the theca layer exit from these cells in order to participate in estrogen biosynthesis, the inference being that extracellular androgens diffuse into the granulosa cell layer. After ovulation, extensive neovascularisation of the follicle takes place providing increased amounts of cholesterol to the luetinized granulosa cells allowing these cells to secrete increased quantities of progesterone during the luteal phase of the menstrual cycle.

Functions of steroid hormones

The steroid hormones are synthesized mainly by endocrine glands such as the gonads (testis and ovary), the adrenals and (during gestation) by the fetoplacental unit, and are then released into the blood circulation. They act both on peripheral target tissues and the central nervous system (CNS). An important function of the steroid hormones is to coordinate physiological and behavioral responses for specific biological purposes, e.g. reproduction. Thus, gonadal steroids influence the sexual differentiation of the genitalia and of the brain, determine secondary sexual characteristics during development and

sexual maturation, contribute to the maintenance of their functional state in adulthood and control or modulate sexual behaviour. It has been discovered that in addition to the endocrine glands, the CNS is also able to form a number of biologically active steroids directly from cholesterol (the so-called "neurosteroids"). These neurosteroids, however, are more likely to have "autocrine" or "paracrine" functions rather than true endocrine effects.

Steroid metabolism

Despite their relatively simple chemical structure, steroids occur in a wide variety of biologically active forms. This variety is not only due to the large range of compounds secreted by steroid-synthesizing tissues, but also to the fact that circulating steroids are extensively metabolized peripherally, notably in the liver, and in their target tissues, where conversion to an active form is sometimes required before they can elicit their biological responses (Fig 7). Steroid metabolism is therefore important not only for the production of these hormones, but also for the regulation of their cellular and physiological actions.

Hepatic biotransformation of steroids

The liver plays an important role in maintaining homeostasis in all vertebrates. In addition to metabolizing toxins, the liver plays a key role in hormone homeostasis, as it metabolizes both peptide and steroid hormones. Hepatic metabolism of many steroids and toxins can occur in a sexually dimorphic pattern and thus can serve as a biomarker for exposure to both naturally occurring and synthetic hormones. The control of steroid hormone profiles involves several variables, including rate of hormone synthesis, interactions among hormones, and rates of secretion, transport, biotransformation and elimination. One route through which normal control could be disrupted is xenobiotic induction of sex steroid metabolizing cytochrome P-450 enzyme complex. Inducing or blocking these enzymes

Fig 7: Biotransformation of steroids in hypothalamus, pituitary and liver

Hypothalamus and pituitary

Progesterone ----------→ 5α-pregnane 3,20 dione (5α reductase)

5α-pregnane 3,20 dione ----------→ 3α-hydroxy 5α-pregnane 3,20 dione (3α HSDH)

Liver

Oxido reduction

Estrone + NADH + H^+ <==========> Estradiol + NAD (17 β hydroxy steroid oxido reductase)

Glucoronide formation

Steroid-OH + UDPGA-------→ Steroid glucuronide (UDPG Transferase)

Sulphate formation

(1) SO_4^- + ATP --- ------→ APS + P-Pi (ATP sulphurylase)

(2) APS + ATP --- ------→ PAPS (ATP kinase)

(3) Steroid-OH + PAPS --- ------→ Steroid-O-SO^{--} + PAP + H+ (ATP sulphokinase)

conceivably alters the natural balance of circulating sex steroids. Several mechanisms are used for hormone biotransformation in the liver. Direct conjugation, in which the steroid is conjugated to glucuronic acid or sulphate, produces a more water-soluble product that can be excreted in urine (de Bethizy and Hayes, 1994). Steroid hydroxylation accomplishes the same goal by stereo-selectively and regio-specifically attaching hydroxyl groups to a steroid (Wilson et al., 1998). Oxido-reduction of testosterone to androstenedione, dihydro-testosterone and androstanediols is another hepatic biotransformation pathway that influences circulating concentrations of testosterone and other androgens.

Formation of steroid conjugates

Conjugation (formation of hydrophilic molecules) is an important step in steroid catabolism. Most excretory products are in conjugated form. Two major pathways are used:

(1) Formation of glucuronides. This reaction requires uridine diphosphoglucuronic acid (UDPGA) and a glucuronyl transferase. Glucuronic acid is attached to a HO-group on the steroid molecule.

(2) Formation of sulphates. This conversion is catalysed by sulphokinases, which occur in the cytosol of liver, testicular, adrenal and fetal tissues. The substrates are steroids with an HO-group and phosphoadenosine-5`-phosphosulphate (PAPS). This is a three-step reaction which requires magnesium (Mg++) ions.

In addition to being excretory products, sulphates are also found in endocrine tissues and/or the plasma as precursors for hormone synthesis. This is the case of dehydroepiandrosterone sulphate (DHEAS), which is used notably for estrogen biosynthesis in the fetoplacental unit. Sulphatases occurring in the microsomal fraction of

liver, testis, ovary, adrenal and placenta catalyze the hydrolysis of sulphated steroids to free steroids.

Effect of neurotransmitters and neuromodulators on H-P-G axis

Serotonin: Serotonin mediates the influence of short photoperiod on reproduction in temperate mammalian species: darkness stimulates the production of hypothalamic and/or pineal indoleamines, which via the hypothalamic suprachiasmatic nucleus and pars tuberalis inhibit hypothalamic GnRH production and release. They thereby regulate gonadotropin output. It has been observed that serotonin injection increases the LH and FSH uptake by the gonadotropin receptor on rat ovaries (Trentini et al., 1976). *In vitro* experiments strongly demonstrated direct effects: it stimulates progesterone and estradiol secretion by cultured rat preovulationary follicles (Tanaka et al., 1993) and human granulosa cells (Bodis et al., 1992), estradiol output by hamster preovulationary follicles (Terranova et al., 1990) and progesterone release by isolated porcine granulosa (Sirotkin 1995) and bovine (Rhodes and Randell, 1982) but not baboon (Khan-Dawood and Dawwod, 1993) luteal cells. Further more, serotonin treatment stimulates oxytocin and cGMP and inhibits vasopressin and cAMP output by porcine granulosa cells (Sirotkin 1995).

Catecholamines

Dopamine can act both as a neurotransmitter and as a hormone. Dopamine is released from synaptic vesicles to effect nerve cells and is released by the hypothalamus into the portal blood system of the pituitary to primarily inhibit the activity of the acidophilic cells of the anterior pituitary in their production of prolactin. Dopamine also inhibits LH, FSH, and TSH production in the basophilic cells. Dopamine has a less clear role but is thought to

slightly reduce GnRH secretion. Norepinephrine exhibits stimulatory effects on LH secretion and appears to be dependant on the presence of ovarian steroids, particularly estradiol. Epinephrine is more potent than norepinephrine in stimulating GnRH secretion, which occurs at the ovulatory LH surge.

Endogenous opioid peptides such as endorphin, enkaphelin and dynorphin exert an inhibitory influence on GnRH secretion and are involved in the steroid regulation of GnRH release in women

Neuropeptide Y

Similar to "classical" neurotransmitters (e.g., its frequent coexistence partner. norepinephrine), neuropeptide Y plays an obligatory role in the preovulatory LH discharge in young rats by potentiating the action of GnRH on LH release at the level of the pituitary. A deficit in neuropeptide Y secretion and action in the hypothalamus is responsible for the absence of LH surge in old rats.

Galanin

Galanin is a neuropeptide, which is not a member of any known family of neuropeptides, despite repeated efforts to discover related peptides. It is widely expressed in the brain and thought to play an important role in the regulation of neuroendocrine processes. Its actions are mediated via Gi-protein-coupled receptors and ion channels, usually producing inhibition of secretion of a transmitter or hormone in the nervous and endocrine system (Splett et al., 2003). Galanin mRNA levels rise in the pituitary during pregnancy, and they are also present in the placenta. During the estrous cycle of the rat, pituitary galanin mRNA levels vary 30-fold. The regulation of the galanin gene, tissue levels of galanin in the pituitary, as well as the regulation of circulating galanin levels. is under strong estrogen

control in the female rat. Galanin inhibits dopamine release from the median eminence and strongly stimulates the release of prolactin and growth hormone in humans and in rats. Recently, a new member of this neuropeptide family, which shares some sequence homology with galanin, was isolated from the porcine brain—**galanin-like peptide (GALP)**. It was found that the expression of GALP in the arcuate nucleus is regulated by the adipocyte-derived hormone leptin, and that GALP plays a role in the neuroendocrine regulation of pituitary function (Matsumoto et al., 2000).

NO

NO is a gaseous intracellular transmitter in the CNS produce by a NO synthase (NOS). which converts L-arginine to L-citrulline. The NOS neuronal form (NOS-1), which is constitutively expressed, is regulated by Ca/Calmodulin and is dependent on oxygen and NADPH as co-substrate. In rats, NO participate in both the control of sexual behavior and GnRH/LH release. NOS is not found in GnRH neurons, although their neurons are often surrounded by NOS cells. NOS synthesis is steroid dependent and estrogen receptor alpha has been found in NO producing neurons of the preoptic area and hypothalamus (Dufourny and Skinner, 2002). NO has been implicated in stress regulation. NOS mRNA is increased in the paraventricular nucleus and anterior pituitary following stress in rats and it has been suggested that NO acts at the hypothalamic level to modulate the physiological response to stresses.

Leptin

Leptin has been shown to be an important satiety signal and metabolic activator. Leptin has a stimulatory effect on the reproductive system, which may serve as a conduit linking the body's metabolic and reproductive control systems. Emerging evidence in several species

Oxytocin

The addition of oxytocin to cultures of pituitary cells from female rats elicited a concentration-dependent secretion of LH (Evans & Tulloch 1995). This secretion was enhanced in an oestrogenised environment and was inhibited by progesterone and testosterone. Conversely oxytocin receptor antagonist suppressed the production of the LH surge in a dose-dependent manner, indicating that endogenous oxytocin is a crucial component of LH regulation. In the human female, oxytocin administered during the late follicular phase advanced the onset of the midcycle LH surge. Furthermore rats administered oxytocin on pro-oestrus had higher LH pituitary content (Evans & Tulloch 1995). Thus oxytocin promoted synthesis and replacement in the pituitary of LH released into the circulation. Incubation of pituitary pieces with oxytocin plus GnRH induced greater LH than released by oxytocin and GnRH separately (Evans et al., 1995). Thus oxytocin synergised with GnRH in stimulating LH release. Perifusion studies performed on hemipituitaries demonstrated that a LH response could be determined by the presence of three peptides, oxytocin, neuropeptide Y and GnRH (Evans, 2002). Hence oxytocin is potentially involved also in multiple interactions during the process of LH regulation. LH regulation is therefore apparently the result of a community of peptides acting in a co-operative network.

Endocrine changes in pregnancy

Pregnancy

The period of gestation in humans is approximately 40 weeks from the last menstruation. Sperm that are deposited in the female reproductive tract remain viable for several days; the ovum is contract only for approximately one day. The best time for intercourse to lead

to successful fertilization is on or before ovulation. During that time in the female reproductive tract the sperm undergo a final stage of maturation called capitation. Fertilization usually takes place in the oviducts and during its passage to the uterus the zygote begins to divide so that by the time it reaches the uterus (3 days) the cellular mass is called a morula. At this stage should the morula divide and separate, identical twins are formed. The morula develops into the blastocyst, which comprises an outer trophoblastic layer, which forms part of the chorion and an inner mass of embryonic cells. Implantation into the wall of the uterus takes about 7 days after fertilization and within two days the trophoblastic layer of the blastocyst is producing a hormone called human chorionic gonadotrophin (hCG - early detection of this hormone can be used as a test for pregnancy). The hCG is critical for the maintenance of the corpus luteum which is now able to continue to produce oestrogen and progesterone. These two hormones are necessary for the continuation of pregnancy during the first three months of foetal life. After three months the placenta supersedes the corpus luteum's role. Therefore the placenta is not only a medium for nutrient / waste product and respiratory gaseous exchange but also an endocrine gland. The placenta takes over much of the role of the ovaries after three months (first trimester- third division) providing peptide and steroid hormones for the maintenance of pregnancy.

The placenta

Pregnancy is associated with several alterations in hormonal balance primarily because the placenta is a pleiotropic endocrine gland and act as a barrier between mother and fetus. The placenta is able to provide at least four hormones necessary for pregnancy to continue. Two of these are polypeptides, hCG (functionally similar to LH) and hCS or human

chorionic somatomammotrophin and two are steroids, progesterone and oestrogen. hCS is produced by the trophoblastic cells and is structurally similar to growth hormone. The levels of this hormone increase through pregnancy and appear to be necessary for the development of the breasts. HCG, a counter part of LH regulates progesterone secretion, which is critical for pregnancy to continue since it maintains the endometrium. The levels of progesterone reach a peak after about 3-4 weeks following fertilization and then decline before increasing again up to the time of parturition. The latter rise is important for the development of the breasts. There are several estrogen hormones secreted by the placental. The placenta relies on the fetus to provide it with precursors for steroid synthesis and thus the functional unit is the feto-placental unit. Like progesterone, estrogen is necessary for the uterus to develop to accommodate the growing fetus and for the latter stages in the development of the breasts.

In rat, progesterone and estrogen is obligatory requirement for intrauterine implantation. Main events that take place in rat are: 1) priming the uterus with the estrogen in proestrus stage, 2) conditioning of uterus with progesterone during first 4 days of the pregnancy, 3) a prenidatory peak of estrogen on day 4 of pregnancy, the day before implantation occurs. Estrogens cause the proliferation of cells of uterine endometrium and vaginal epithelium. It also helps in rhythmic motility of the myometrium. Estradiol has anabolic effects on bone and cartilage and promotes growth. Progestins reduce the proliferative activity of estrogens on the vaginal epithelium, convert uterine epithelium to secretory, increase secretory glands, and increase glycogen content. An increasing level of progesterone is essential for embryonic development to proceed and for subsequent implantation to occur. Progesterone has many functions like anti-inflammatory and immunosuppressive properties, which

protects the conceptus from immunologic rejection by the mother. The nidatory estrogen peak induces a secretory endometrium and a state of receptivity in the uterus towards the decidualisation stimulus of the embryo. This includes inhibition of endocytosis in the luminal epithelium and release of serum and non-serum proteins into uterine lumen, which is maximal at the day of implantation.

Parturition

The mechanism by which labour is initiated is very poorly understood in humans. Oxytocin from the pituitary regulates the expulsive phase of labour and following delivery the contraction of the uterus to reduce blood loss. The corpus luteum produces Relaxin, a large polypeptide hormone which is able to prepare the cervix for delivery in many animals but thought to be of minor importance in humans. The levels of prostaglandins appear to be critical for human parturition since they soften and dilate the cervix and initiate uterine contractions. Indeed, prostaglandin inhibitors are to prevent preterm labour.

Lactation - *the roles of prolactin and oxytocin*

Lactation involves several hormones including the placental hormone hCS (human somatomammotrophin). Lactation occurs after parturition when there is a sudden drop in oestrogen and progesterone levels. Once milk is formed and stored in the mammary gland its release is dependent upon neural signals travelling to the hypothalamus via somatic nerves from the nipple and the spinal cord. The continued production of prolactin is dependent upon continued suckling by the infant, which sends signals to the hypothalamus to inhibit the release of prolactin-release inhibiting hormone (dopamine). The start of normal ovulatory cycles may be disrupted for several months following childbirth and this is probably due to the suckling stimulus inhibiting FSH and LH production. The release of

milk or "milk let-down" is dependent upon oxytocin from the posterior pituitary, which receives direct neural connections from hypothalamic nuclei. The sensory signals from the stimulated nipple activate oxytocin neurons in the paraventricular and supraoptic nuclei that project to the neural lobe. Once activated these neurons release their hormone in to the circulation, carried to its target gland, the breast to cause contraction of the myoepithelial cells. The hormones that are important for the various stages of lactation include; prolactin and hCS - for milk synthesis, removal of placental steroids after birth - lactogenesis or milk synthesis by the alveolar cells with secretion into the alveolar lumen; maintenance of established lactation - (galactopoisis) controlled by prolactin due to suckling by the infant and; milk ejection - controlled by the neurohypophyseal hormone. oxytocin. The release of prolactin from the adenohypophysis is dependent upon the secretion of prolactin releasing substances (VIP, oxytocin) and decreased release of prolactin-release inhibiting substances (dopamine).

Endocrine toxicology

Many chemicals in common use enter the bodies of human, domestic animals, and wildlife, chiefly through contaminated food and water. These chemicals mimic like hormones, body mistakes them for natural hormones and reacts to them in ways that cause deep and permanent trouble, especially when exposure occurs during the critical period of development.

Endocrine-disrupting chemicals mimic hormones, but with one key difference. Natural hormones do their work as messenger (or as stimulant of a cascade of other effects), and then the body disassembles them and removes them from the blood stream. In contrast, when industrial chemicals and pesticides mimic hormones, they do not disappear quickly.

They tend to remain in the body for very long periods, doing the work of hormones at times, and in ways, that are inappropriate and destructive.

Reproductive toxicity

Reproductive toxicity can be defined as "The occurrence of adverse effects on the reproductive system that may result from exposure to environmental agents. Toxicity may be expressed as alterations to the reproductive organs and/or the related endocrine system. Developmental toxicity can be defined as the occurrence of adverse effects on the developing organism that may result from exposure before conception (either parent) during prenatal development, or postnatal till the time of sexual maturation. Adverse developmental effects may be detected at any point on the life span of the organism". Developmental toxicity can include fetal death, structural abnormalities or birth weights and functional deficiencies or altered growth.

Reproductive health represents one of the major aspects of human life. The magnitude of occupational and environmental reproductive and developmental health risks of modern society is not well documented. Scientific epidemiological and toxicological data concerning the reproductive and developmental health risks of many chemicals, physical agents and biological agents are limited and in some instances nonexistent. Reproductive toxic effects have unique characteristics from the patterns of organ related toxicity, which are limited to a single organ system. The target of toxicity can include either parent or the offspring. Characteristics, which distinguish reproductive toxicity from other toxic effects.

-- Adverse effects in exposed person become manifest in the other party. For example, exposure to a reproductive toxicant in a male or female may produce an effect in the

conceptus.

-- Infertility may not be evident until children are desired and may therefore go unnoticed for long periods;

-- Normal reproductive function is only expressed intermittently. Disturbances of the reproductive process from occupational reproductive hazards can produce broad range of potential toxic effects.

Heavy metals

Metals are indispensable for life. Some metals are essential to normal metabolic function as trace elements. Of these some are rarely toxic, even at relatively high levels of exposure. Beside sodium, potassium, calcium and magnesium, which sustain the internal physiological balance, there are approximately a dozen other trace elements, which have proven essential. Some are less benign at high levels of exposure; manganese, although essential in trace amounts, can cause Parkinson's like syndrome at high levels and selenium has been linked to an increased risk of cancer. Some metals such as lead and cadmium, which are called **heavy metals**, have no known functions in cells, but have established toxic effects. Heavy metals are present in the environment because of the application of modern technology in the industry and through the scientific and technical advances. The functional, morphological and biochemical effects of these elements manifest themselves at different levels; the organism, organs and tissues, the cell and even at the subcellular level. The biological properties of heavy metals are discussed in terms of three important characteristics: the ability to form irreversibly complexes and chelates with organic ligands, which influence greatly the dynamics of transport, distribution and excretion of several important metal cations; the properties to form organic-metallic bonds

and the potential to undergo oxidation-reduction reactions. Field and laboratory studies indicated that bioaccumulation of heavy metals, occurs in primary and secondary consumers of the food web. Among them lead and cadmium have been shown to accumulate in various tissues such as kidney and the liver. Also their accumulation in other organs as the hypothalamus, pituitary or gonads was reported (Lafuente et al., 1999; Lorenson et al., 1983; Ronis et al., 1998; Paksy et al., 1990).

LEAD

Identity

Lead is the commonest of the heavy elements, accounting for 13 mg/kg of the earth's crust. The oxidation state of lead is +2. Several stable isotopes of lead exist in nature, including, in order of abundance, 208Pb, 206Pb, 207Pb, and 204Pb.

Physicochemical properties	**Property Value**
Physical state-	Soft metal
Melting point-	327 °C
Density	11.34 g/cm3
Boiling point	1740°C at 100 kPa
Solubility	Soluble in dilute nitric and concentrated sulfuric acids

Exposure pathways

Lead, a naturally occurring element that has been used almost since the beginning of civilization, is ubiquitous in the environment today. All human beings have lead in their body, primarily as a result of exposure to manmade sources. The most important pathways are ingestion of chips from lead painted surfaces, inhalation of lead from automobile emissions, food from lead soldered cans, drinking water from lead soldered plumbing and

medications in the form of folk remedies (Table 1). Although inhalation of lead from gasoline is no longer considered as public problem, the lead from dusts in automobile emissions has been deposited in the soil.

Kinetics and metabolism

Adults absorb approximately 10% of the lead contained in food; the gastrointestinal absorption of lead from ingested soil and dust by children has been estimated to be close to 30%. Absorption is increased when the dietary intakes of iron or calcium and phosphorus are low. Iron status is particularly important. Gastrointestinal absorption after ingestion is influenced by the physiological state of the exposed person (e.g. age, fasting, nutritional calcium and iron status) and the physical-chemical properties of the lead ingested (e.g. particle size, mineralogy, solubility, lead species) (ATSDR, 1999). The absorption of lead from ingested soil is less than that from dissolved lead. From ingested soil, 26% is absorbed during fasting, while only 2.5% is absorbed with a meal. Inorganic lead forms complexes with a variety of protein and non-protein ligands. Most of the total body burden of lead is found in the bones. Based on a study of 32 diseased smelter workers, the major soft-tissue organs with lead accumulation were, in descending order, liver, kidney, lungs and brain. A similar pattern of distribution was found in animal studies with rats and mice (ATSDR. 1999).

Table 1: Human exposure to various lead sources

Medium	Rural	Urban	Near point source
Ambient air ($\mu g / m^3$)	0.1	0.1-0.3	0.3-3.0
Indoor air ($\mu g / m^3$)	0.03-0.08	0.03-0.2	0.2-2.4
Soil (ppm)	5-30	30-4,500	150-15000
Street Dust (ppm)	80-130	100-5000	25,000
House Dust (ppm)	50-500	50-3,000	100-20,000
Diet (ppm)	0.002-0.08	0.002-0.08	0.002-0.08
Drinking water ($\mu g/L$)	5-75	5-75	5-75
Paint (mg/cm^2)	<1 --- >5	<1 --- >5	<1 --- >5

The principal vehicle for the transport of lead from the intestine to the various body tissues is the red blood cell, in which lead is bound primarily to haemoglobin and has a special affinity for the β, δ and, in particular, fetal γ chains. Following its absorption, lead appears both in a soft tissue pool, consisting of the blood, liver, lungs, spleen, kidneys, and bone marrow, which is rapidly turned over, and in a more slowly turned over skeletal pool. The half-life of lead in blood and soft tissues is about 36-40 days for adults, so that blood lead concentrations reflect only the intake of the previous 3-5 weeks. In the skeletal pool, the half-life of lead is approximately 17-27 year. In adults, some 80-95% of the total body burden of lead is found in the skeleton, as compared with about 73% in children (Alessio and Foa, 1983). The blood lead concentration can therefore be used as a reasonably good indicator of exposure from all sources (Mushak P et al, 1989).

The foetal/maternal Pb blood concentration ratio was approximately 0.9. One of the sources of maternal lead is the bones, which are catabolised for the production of the foetal skeleton; another may be the increased absorption during pregnancy. Transfer of maternal lead to offspring can also occur during breast-feeding. The mother animals were exposed prior to or during lactation, and around 25-33% of the maternal dose was transferred to the sucklings (ATSDR, 1999). In humans, the concentration of lead in breast milk was 10-30% of the maternal blood Pb level (WHO, 1995). Placental transfer of lead occurs in humans as early as 12 weeks of gestation, and uptake of lead by the fetus continues throughout development (Barltrop D. 1969). The concentration of lead in umbilical cord blood is 80-100% of the maternal blood lead level; the same applies to blood lead in the fetus (Angell and Lavery, 1982; Gershanik et al., 1974).

Inorganic lead is not metabolized in the body. Unabsorbed dietary lead is eliminated in the

faeces, and lead that is absorbed but not retained is excreted unchanged via the kidneys or through the biliary tract. Metabolic balance studies in infants and young children indicated that mean dietary intakes of 3-4 μg/kg of body weight per day did not cause retention of lead (Moore et al., 1982). The rate of disposition of particulate airborne lead in adult humans is approximately 30-50%, and is modified by factors such as particle size and ventilation rate.

WHO reports that 60% of absorbed lead is retained in the body and 40% is excreted (WHO, 1995). Davidson (1994) states that approximately 75% of inorganic lead absorbed into the body is excreted in urine and less than 25% is excreted in faeces.

Acute/Repeated/chronic toxicity

The term 'acute toxicity' is used to describe the adverse effects on health, which may result from a single exposure to a substance via ingestion, dermal contact or inhalation. Repeated dose toxicity comprises the adverse general toxicological effects (i.e. excluding reproductive, genotoxic or carcinogenic effects occurring as a result of repeated daily exposure to a substance for a part of the expected life span, sub-acute or sub-chronic exposure or, in the case of chronic exposure, for the whole life span or the major part of the life span. In any case, the toxic effects of lead are independent of the route of exposure, and correlated with the absorbed dose as expressed in the blood Pb level.

Effects on humans

There is a very substantial database on human health effects of lead. These health effects are, in general, related to the absorbed dose of lead, as expressed in the blood Pb level, and are mostly a consequence of multi-route exposure. The toxic effects of lead are independent of the route of exposure, and are correlated with the internal exposure

expressed in the blood Pb level. There is no absolutely equivalent animal model for the effects of lead on humans. Animal studies are still used, as most of the time no human data will be available, when available, the nature, duration and level of exposure are usually not well known.

Guideline value

The guideline values for human health risk standard with regard to exposure to lead are given in Table 2.

Neurological effects

The most sensitive target of lead poisoning is the nervous system. Several lines of evidence demonstrate that both the central and peripheral nervous systems are the principal targets for lead toxicity. The effects include subencephalopathic neurological and behavioural effects in adults, and there is also electrophysiological evidence of effects on the nervous system of children at blood lead levels well below 30 μg/dl. In addition to the lack of a precise threshold, childhood lead toxicity may have permanent effects. Primary school children with high tooth lead levels but with no known history of lead poisoning had larger deficits in psychometric intelligence scores, speech and language processing, attention, and classroom performance than children with lower levels of lead (Needleman et al., 1979). Adults also experience CNS effects at relatively low blood lead levels, manifested by subtle behavioral changes, fatigue, and impaired concentration. Peripheral neuropathy with mild slowing of nerve conduction velocity has been reported in asymptomatic lead workers. The lowest doses that can cause disturbances in nervous system are given in the Table 3.

Table 2: Limit values for lead

Medium	Lead species	Limit name	Limit value	Regulatory authority	Source
General population					
Air	lead	guideline value	0.5-1.0 ug/m3	WHO	WHO. 1995
Drinking Water	lead	guideline value	0.01 mg/l	WHO	WHO. 1996
n/a	lead	PTWI	25 ug/kg bw	JECFA	WHO. 1996
Workers					
Air	lead	OEL-TWA	0.15 mg/m3	EU	EU, 2001
Air	lead and inorganic compounds	TLV-TWA	0.05 mg/m3	ACGIH	ACGIH, 1999 & 2000
Blood	lead	(proposed) BAT	30 ug/dl	DFG	DFG, 2000
Blood	lead	(women<45 years) (proposed) BAT	40 ug/dl	DFG	DFG, 2000
Blood	lead and compounds	(other workers) Action level	25 ug/dl	HSE	HSE, 1999
Blood	lead and compounds	(women of rep. age) Action level	40 ug/dl	HSE	HSE, 1999
		(persons under 18)			

PTWI- Provisional Tolerable Weekly Intake
OEL-TWA- Occupational Exposure Limit-Time Weighted Average
BAT- Biological working material value

Table 3: Lowest observed adverse effect levels (LOAELs) for lead induced nervous effects

Blood Pb level (ug/l)	Source	Adults	Children
1000-1200	SCOEL, 2000	Encephalopathic signs and symptoms	
800-1000	SCOEL, 2000		Encephalopathic signs and symptoms
≥400	SCOEL, 2000	Neurobehavioral effects	
≥110	ASTDR, 1999		Neurobehavioral effects
≥500	ASTDR, 1999	Overt subencephalopathic neurological symptoms, cognition impairment	
200-300	ASTDR, 1999	Peripheral nerve dysfunction (slowed nerve conduction velocities)	

Table 4: Lowest observed adverse effect levels (LOAELs) for lead induced hematological effects

Lead level	Reference	Adults	Children
800-1000	Davidson, 1994	Frank Anemia	--
700	Davidson, 1994		Frank Anemia
500	WHO, 1987	Reduced hemoglobin synthesis	
400	WHO, 1996	Increased urinary ALA, elevated coproporphyrin	Increased urinary ALA, elevated coproporphyrin
200-300	WHO, 1996	FEP elevation in males	Reduced hemoglobin synthesis, FEP elevation
150-200	WHO, 1996	FEP elevation in females	

Hematological effects

Lead interferes with the activity of several of the major enzymes involved in the biosynthesis of haem. The only clinically well-defined symptom associated with the inhibition of haem biosynthesis is anaemia (Moore, 1988), which occurs only at blood lead levels in excess of 40 µg/dl in children and 50 µg/dl in adults (WHO, 1987). Lead can induce two types of anemia. Acute high-level lead poisoning has been associated with hemolytic anemia. In chronic lead poisoning, lead induces anemia by both interfering with erythropoiesis and by diminishing red blood cell survival. Anemia is not an early manifestation of lead poisoning and is evident only in prolonged periods of lead exposure.

Enzymes involved in the synthesis of haem include δ-aminolaevulinate synthetase whose activity is indirectly induced by feedback inhibition, resulting in accumulation of d-aminolaevulinate, a neurotoxin and δ-aminolaevulinic acid dehydratase (δ-ALAD), coproporphyrinogen oxidase, and ferrochelatase, all of whose activities are inhibited (EPA, 1986). The activity of δ-ALAD is a good predictor of exposure at both environmental and industrial levels, and inhibition of its activity in children has been noted at a blood lead level as low as 5 µg/dl (Granick et al, 1973); however, no adverse health effects are associated with its inhibition at this level.

Inhibition of ferrochelatase by lead results in an accumulation of erythrocyte protoporphyrin (EP), which indicates mitochondrial injury (Mushak et al., 1989). Thus lead interferes with amino acid incorporation causing increased fragility of the RBC and consequently an increased rate of destruction. The mechanism for shortened erythrocyte survival is not well understood. But it has been demonstrated that an inhibition of erythrocyte membrane Na K ATPase occurs in people with moderately elevated lead

exposure. Leakage of potassium thus leads to an increased mechanical fragility of the cell. The lowest doses that can cause hematological effects are given in the Table 4.

Renal Effects

Renal disease has long been associated with lead poisoning; however, chronic nephropathy in adults and children has not been detected below blood lead levels of 40 μg/dl (Campbell et al., 1977). Acute proximal tubular dysfunction is characterized by the appearance of prominent inclusion bodies of a lead-protein complex in the proximal tubular epithelial cells at blood lead concentrations of 40-80 μg/dl (Ritz et al., 1988).

There are indications of increased hypertension and lead induced renal insufficiency at blood lead levels greater than 37 μg/dl (Harlan et al., 1985; Pirkle et al., 1985). The lowest doses that can cause renal effects are given in the Table 5.

Carcinogenic and mutagenic effects

Substances or preparations are defined as carcinogenic if they induce cancer or increase its incidence after inhalation, ingestion or dermal absorption. Occupational exposure to lead is associated with increased mitotic activity in peripheral lymphocytes, increased rate of abnormal mitosis and increased incidence of chromosomal aberrations and sister chromatid exchange, at blood Pb levels ranging from 22 to 89 μg/dl. Cytogenetic studies in humans exposed to lead (blood lead levels >40 μg/dl) have given conflicting results; chromatid and chromosomal aberrations, breaks, and gaps were reported in 9 of 16 studies but not in the remainder (IARC, 1987). Two cases of renal cancer have been reported in workers exposed to lead with symptoms of lead poisoning and high blood Pb levels. In at least one of these cases, the tumour contained a high level of lead and had histopathological characteristics similar to those of kidney tumours provoked by lead in

Table 5: Lowest observed adverse effect levels (LOAELs) for lead induced renal effects

Dose	Reference	Adults	Children
≥400	WHO, 1996	Chronic nephropathy	Chronic nephropathy
1000-1200	WHO, 1996	Muscle tremor, kidney damage	
800-1000	WHO, 1996		Muscle tremor, kidney damage
400-800	Ritz et al., 1998	Proximal tubular dysfunction	
≥370	Pocock et al., 1984	hypertension	

animals (ATSDR, 1999). According to WHO (1995), which used essentially the same study database, there is no association between renal cancer and lead exposure. A similar conclusion is reached by Davidson (1994). Both the EPA (2001) and IARC (1987) consider the database inadequate to evaluate carcinogenic risks of lead and inorganic lead compounds to humans. Recent data on cancer risks of workers exposed to lead would probably justify a re-evaluation in the near future (SCOEL, 2000).

Effects on laboratory animals and in vitro test systems

Neurological effects

Research on young primates has demonstrated that exposure to lead results in significant behavioural and cognitive deficits, e.g. impairment of activity, attention, adaptability, learning ability, and memory, as well as increased distractibility. Such effects have been observed following postnatal exposure of monkeys to lead for 29 weeks in amounts resulting in blood lead levels ranging from 10.9 to 33 µg/dl (Rice, 1987). These effects persisted into young adulthood, even after levels in the blood had returned to 11-13 µg/dl, and were maintained for the following 8-9 years (Gilbert and Rice, 1987). Studies on small groups of monkeys dosed continuously from birth onwards with 50 or 100 µg/kg of body weight per day showed that at 7-8 years of age there were still significant deficits in both short-term memory and spatial learning (Rice and Karpinski, 1988).

In vitro tests on human cells were positive for chromosomal damage in one case and negative in two others. *In vivo* short-term tests on mice, rats, cattle, and monkeys were positive in three cases (dominant lethal test and chromosome damage to bone marrow cells) but negative in five others (IARC, 1987). *In vivo* studies with mice, rats and monkeys did not provide evidence for clastogenic properties of lead. However, one chronic

study with monkeys showed severe chromosome aberrations occurred as a consequence of lead exposure with cadmium-deficient diet (ATSDR, 1999). WHO (1995) concluded on the *in vivo* and *in vitro* studies, that lead is a weak mutagen in mammalian systems, but a strong mitogen.

The available data on carcinogenicity following ingestion of lead by laboratory animals indicate that lead acetate and phosphate are carcinogenic, and that the most common tumour site is the kidney. In rats, no evidence of renal tumours was found at doses below 200 mg/l in drinking water (WHO, 1995). IARC (1987) and the EPA (2001) consider inorganic lead to be an animal carcinogen. Renal tumours (mostly tubular epithelial adenomas) developed in male rats at 500, 1000, and 2000 mg/kg, but only at 2000 mg/kg in female rats.

Reproductive effects

Reproductive effects on in human and animals have been discussed later.

Cadmium

Cadmium is a naturally occurring metallic element, one of the components of the earth's crust and present everywhere in our environment. Its existence was revealed in 1817. It owes its name to "cadmia fornacum ", the "zinc flowers " which formed on the walls of zinc distillation furnaces.

Its industrial applications were developed, particularly during the first half of the 20th century, based on its unique chemical and physical properties.

Identity

Cadmium is a metal with an oxidation state of +2. It is chemically similar to zinc and occurs naturally with zinc and lead in sulfide ores.

Physicochemical properties	Property Value
Physical state	Soft white solid
Density	8.64 g/cm3
Melting point	320.9 °C
Boiling point	765 °C at 100 kPa
Solubility	Soluble in dilute nitric and concentrated sulfuric acids

Exposure pathways

Cadmium is present in ambient air in the form of particles in which cadmium oxide is probably an important constituent. Cigarette smoking increases cadmium concentrations inside houses. The average daily exposure from cigarette smoking (20 cigarettes a day) is 2–4 μg of cadmium (Ros and Slooff, 1987). Cadmium concentrations in unpolluted natural waters are usually below 1 μg/litre. Contamination of drinking-water may occur as a result of the presence of cadmium as an impurity in the zinc of galvanized pipes or cadmium-containing solders in fittings, water heaters, water coolers, and taps. Levels of cadmium could be higher in areas supplied with soft water of low pH, as this would tend to be more corrosive in plumbing systems containing cadmium.

Food is the main source of cadmium intake for nonoccupationally exposed people. Crops grown in polluted soil or irrigated with polluted water may contain increased concentrations and meat from animals grazing on contaminated pastures (IARC, 1976). Animal kidneys and livers concentrate cadmium. Levels in fruit, meat, and vegetables are usually below 10 μg/kg, in liver 10–100 μg/kg, and in kidney 100–1000 μg/kg. In cereals, levels are about 25 μg/kg wet weight. Various sources of exposure are given in Table 6.

Table 6: Human exposure to various cadmium sources

Phosphate Fertilizers	41.3 %
Fossil fuel combustion	22.0 %
Iron & steel production	16.7 %
Natural sources	8.0 %
Non-ferrous metals	6.3 %
Cement production	2.5 %
Cadmium products	2.5 %
Incineration	1.0 %

Kinetics and metabolism

Cadmium is more efficiently absorbed from the lungs than from the gastrointestinal tract (ATSDR, 1989). The absorption efficiency depends on solubility of the specific cadmium compound as well as its exposure concentration and route. Actual cadmium absorption via inhalation exposure has been estimated to be 30 to 60% in humans (Friberg et al., 1974; Elinder et al., 1976).

Inhalation is dependent upon the particle size >= 10μm diameter tend to be deposited in the upper airways and particles <= 0.1 μm diameter are deposited in the alveolar region. Alveolar deposition efficiency in animal models ranges from 5 to 20% (Barrett et al., 1947; Boisset et al., 1978). Based on physiological modeling, cadmium deposition in the alveolar region of humans was estimated to be up to 50% for small particles (Nordberg et al., 1985). Absorption of cadmium from the gastrointestinal tract appears to be a saturable process with the fraction absorbed decreasing at high doses (Nordberg et al., 1985). It is also important to distinguish true absorption from simple retention of cadmium in the microvilli of the small intestine (Foulkes et al., 1986). In healthy persons 3–7% of the cadmium ingested, is absorbed; in iron-deficient people, this figure can reach 15–20% (Krajnc et al., 1987). Also cadmium absorption may be decreased by divalent and trivalent cations (Zn+2, Mg+2, Cr+3), and increased by iron and calcium deficiencies (Flanagan et al., 1978; Foulkes et al., 1986; Goyer, 1991). Indeed, recent studies (Vahter et al. 1996) have suggested that overall nutritional status is more important determinant of cadmium uptake than is the actual amount of cadmium ingested.

Cadmium is transported in the blood by red blood cells and high-molecular-weight proteins such as albumin (Goyer, 1991). Normal blood cadmium levels in adults are <

1μg/dL. Although cadmium is widely distributed throughout the body, 50 to 70% of the body burden accumulates in the kidneys and liver (Goyer, 1991). As with most metallic elements, there is little or no direct metabolic conversions of cadmium, but rather binding to various biological components, such as protein and nonprotein sulfhydryl groups and anionic groups of various macromolecules (ATSDR, 1989). The principal route of excretion is via the urine, with average daily excretion for human being about 2 to 3 μg (ATSDR, 1989). Daily excretion represents only a small percentage of the total body burden, which accounts for the 17 to >30 years half-life of cadmium in the body (Tsuchiya et al., 1972; Friberg et al., 1974). After binding to metallothionein, it is filtered in the kidney through the glomerulus into filtrate, and then reabsorbed in the proximal tubular cells, where the cadmium–metallothionein bond is broken. The unbound cadmium stimulates the production of new metallothionein, which binds cadmium in the renal tubular cells, thereby preventing the toxic effects of free cadmium. If the metallothionein-producing capacity is exceeded, damage to proximal tubular cells occurs, the first sign of this effect being low-molecular-weight proteinuria.

Tissue cadmium concentrations increase with age in a linear fashion till 50-60 years of age. Both kidney and liver act as cadmium stores; 50–85% of the body burden is stored in kidney and liver, 30–60% being stored in the kidney alone. The biological half-life in humans is in the range 10–35 years. About 0.007% of the body burden is excreted daily by adults. but individual variation is large. Unabsorbed cadmium is removed from the gastrointestinal tract by fecal excretion. Typical daily cadmium excretion has been reported to be about 0.01% of the total body burden (ATSDR, 1989). There is some evidence for biliary excretion of cadmium (Klaassen et al., 1978). There is evidence that the placenta is

a partial barrier to cadmium, and that the fetus is exposed to only small amounts of maternal cadmium (ATSDR, 1989).

Acute/ Sub acute/chronic toxicity

Depending on the severity of exposure, clinical signs of cadmium poisoning following acute exposure include: nausea, vomiting, abdominal cramps, headache, muscle cramps, exhaustion, shock, and death (USAF, 1990). Severe exposure may result in pulmonary edema and death (USAF, 1990). If the pulmonary edema is resolved, late-occurring kidney and/or liver damage may develop. Friberg et al. (1974) indicated that exposure to 1 mg Cd/m3 for 8 hours is "immediately dangerous to humans" and the World Health Organization (WHO, 1980) identified 0.5 mg Cd/m3 as the threshold for respiratory effects resulting from an 8-hour exposure.

Toxic effects of cadmium are a function of a critical concentration being attained in a target organ. Hence similar effects will occur following long-term exposure to low cadmium levels and short-term exposure to high concentrations (Wang and Foulkes, 1984). Consequently, renal and hepatic toxicity may occur if toxic cadmium levels are attained in these organs even during subacute exposure. Both pulmonary effects (emphysema, bronchiolitis, alveolitis) and renal effects (proteinuria) may occur following subacute inhalation exposure to cadmium and cadmium compounds (ATSDR, 1989).

Effects on humans

It has been well established that excess cadmium exposure produces adverse health effects on human beings. In the past, occupational exposure was a significant contributor to total cadmium intake. The principal determinants of human cadmium exposure today are smoking habits, diet, and, to a certain extent, occupational exposure. Usually 5% of oral

and 30 to 50% of inhaled cadmium is taken up into the blood. Once cadmium enters the body, it is very strongly retained; therefore, even low doses may build up significant cadmium levels in the body if exposure continues for a long time. The amount of cadmium needed to cause an adverse effect in an exposed person depends on the chemical and physical form of the element. In general, cadmium compounds that dissolve easily in water (e.g., cadmium chloride), or those that can be dissolved in the body (e.g., cadmium oxide), tend to be more toxic than compounds that are very hard to dissolve (e.g., cadmium sulfide). Lowest observed adverse effect levels (LOAELs) for cadmium induced health effects are given in Table 7.

Guideline

The World Health Organisation (WHO) has established a provisional tolerable weekly intake (PTWI) for cadmium at 7 μg/kg of body weight This PTWI weekly value corresponds to a daily tolerable intake level of 70 μg of cadmium for the average 70-kg man and 60 μg of cadmium per day for the average 60-kg woman. At an absorption rate of 5% from ingestion, the average person is believed to retain about 0.5 to 1.0 μg of cadmium per day from food. The guideline values for human health risk standard with regard to exposure to cadmium are given in Table 8.

Renal effects

Cadmium mainly accumulates in the kidneys. At high levels it can reach a critical threshold and can lead to serious kidney failure. Cadmium is known to accumulate in the human kidney for a relatively long time, from 20 to 30 years. This kidney disease is usually not life-threatening, but it can lead to the formation of kidney stones. Cadmium affects the resorption function of the proximal tubules, the first symptom being an increase

Table 7: Lowest observed adverse effect levels (LOAELs) for cadmium induced health effects

Dose	Source	Humans	Animals
0.5 mg/m3 for 8 hr.		Respiratory effects	
30 mg CdCl2 for 6 weeks (rats)	Kotsonis and Klassen, 1978		Histological changes in liver
0.6 mg/kg bw/day for 10 weeks (mice)	Koller et al., 1975		Decrease in number of spleen plaque forming cells
20 ug/m3 for 27 years	Materne et al., 1975	Kidney lesions	
0.05 mg/m3 for 6-12 years	Kjellstorm et al., 1977	Proteinuria	
1.2 mg/kg/day for 24 hr	Kotsonis and Klassen, 1978		No renal effects observed

Table 8: Limit values for cadmium in Air and Water

Medium values	EU Limit values	EU Guide values	USEPA Standard	WHO Guide
Air (milligrams per cubic meter)				
Not to be exceeded in rural areas	-	-	0.001	0.005
Not to be exceeded in urban areas			0.01	0.02
Drinking water and surface **water** intended for **drinking** (milligrams per liter)	5	1	10	3

Sources: **Air**: WHO 1987. **Water**: CEC 1975, 1980 (EU **limit** and EU guide); United States. CFR. vol. 21, no. 52 (USEPA); WHO 1993.

be associated with an increased incidence of respiratory tract cancer (ATSDR, 1989).

Other effects

Cadmium at high doses is also known to produce health effects on the respiratory system and has been associated with bone disease. The effects on the skeleton are painful and debilitating. Cadmium exposure can also promote hypertension and heart disease.

Effects on laboratory animals and *in vitro* test systems

Cadmium compounds have a moderate acute oral toxicity; oral LD50 values for mice and rats range from 60 to over 5000 mg/kg of body weight. Major effects are desquamation of epithelium, necrosis of the gastric and intestinal mucosa, and dystrophic changes of liver, heart, and kidneys (Krajnc et al., 1987). After repeated oral administration, the critical effect in animals is a characteristic lesion of the proximal tubules in the kidneys resulting in impaired tubular resorption and consequent urinary excretion of low-molecular-weight proteins. In rhesus monkeys, a No-Observed-Adverse-Effect Level (NOAEL) of 3 mg of cadmium per kg of diet (given as cadmium chloride) was found for these effects, which were also produced by repeated oral administration to rats of dose 10 mg of cadmium per litre in drinking water. Effects on bone (osteoporosis), liver, haematopoietic system, and immune system have also been reported at the same dose (Krajnc et al., 1987).

Both negative and positive results have been noted with regard to DNA degradation, decreased fidelity of DNA synthesis, DNA repair, gene mutations, and chromosomal abnormalities in mammalian cell cultures, higher plants, and intact animals. It should be noted that the positive results were often weak and seen at high concentrations that also caused cytotoxicity (Krajnc et al., 1987).

An oral carcinogenicity study in rats with cadmium chloride (1–50 mg of cadmium per kg

of diet) did not reveal significantly increased tumour incidences. No increase in tumour incidence was seen with long-term oral exposure in rats (Krajnc et al., 1987). Lung tumours were induced in rats following the inhalation of inorganic cadmium compounds (Oldiges et al., 1989). Chronic exposure of rats to cadmium chloride aerosols (12.5, 25, or 50 μg/m3) produced a dose-related increase in the frequency of primary lung carcinomas (Takenaka et al., 1983).

Effects of lead and cadmium on reproductive system

The metal toxicants can affect the female reproductive system at multiple sites. This does not imply however that there exists an absolute one to one relationship between a particular measurement and the associated site of action. These sites include the neuroendocrine system, the ovary, accessory sex glands and sexual functions. In context to the regulation of GnRH - LH and FSH - ovaries (as described earlier) lead and cadmium can affect the activity of the hypothalamic-pituitary-gonadal axis.

Effect on central nervous system

Lead

Inorganic lead is known to alter catecholaminergic function in the rat brain. It has been reported that low-level exposure enhances central catecholaminergic functions (Cooper and Manalis, 1983; Nation et al., 1989). Subchronic exposure to low levels of lead result in significant reduction in dopamine (DA) and its metabolites, 3,4-DOPAC and homovanillic acid, in the nucleus accumbens of rats (Jadhav and Kala, 1994; Kala *et al.*, 1994). Also studies have reported that exposure to lead reduces dopaminergic activity in the mesolimbic system (Nation et al., 1989; Laskey, 1992). Consistent with these alterations in the metabolism of DA, the spontaneous and evoked release of DA was found to be altered

by lead (Cooper and Manalis, 1983; Minnema et al., 1988). Kala and Jadhav, 1995 suggested that the decrease in spontaneous release of DA in the lead exposed rats may be due to the effects of lead on decreased availability of DA in the neurons; interference of lead with calcium mediated neurotransmitter release and lead induced alterations in autoreceptor mediated processes.

Cadmium

Cadmium modifies the content of various neurotransmitters in discrete areas of the brain. Shukla and Chandra, (1982) showed changes in dopamine, serotonin (5-hydroxy tryptamine) and norepinephrine (NE) contents in half brain areas after cadmium exposure. Nation et al., 1989 demonstrated modifications in serotonin, 5-Hydroxy indole acetic caid (5-HIA), dopamine and NE contents in the cortex, striatum and hypocampus in rats exposed to cadmium alone or in combination with lead. Lafuente et al., 2000 found a decrease in the 5 HT levels in all hypothalamic regions after both pubertal and post pubertal cadmium exposure accompanied by a marked decrease in 5- HIIA content in the same areas. Considering that serotonin increases both LH and FSH secretion these observations along with the unchanged gonadotropin secretion in pubertal animals exposed to same dose of cadmium indicate the existence of a disruption of the hypothalamic regulatory mechanisms of pituitary hormone secretion. A direct effect of cadmium at the hypophyseal level cannot be excluded since there are some data from the bibliography indicating this mechanism. The role of cadmium as a calcium antagonist has also been reported (Hirning *et al.*, 1988).

Effect on hypothalamic and pituitary hormone secretion

Lead

Various investigators have suggested that the major site of lead action on the HPG axis is at the level of hypothalamus. This view was supported by observations that lead exposed adult male rats demonstrated an impaired release of pituitary LH in response to hypothalamic challenge with the opiate antagonist naloxone, an enhanced pituitary release of LH in response to direct stimulation of the pituitary with LHRH, an enhanced testicular response to hCG, increased pituitary LH stores, and increased GnRH RNA in the hypothalamus (Sokol, 1987; Ronis et al., 1998). In addition Kempinas et al., 1994 reported decrease in LH receptor number in testicular homogenates for adult male rats chronically exposed to lead acetate. A blockade of HPG axis at the hypothalamic level would also be expected to interfere with the negative feedback loop whereby testosterone regulates LH release, since this also occurs largely at the level of the hypothalamus. This probably explains the lack of the reflex increase in plasma LH in the lead exposed animals in response to the drop in plasma testosterone. Oral exposure of female monkeys to a dose of 1 mg Pb/kg bw/day (Pb blood level 35 μg/dl) for a period up to 10 years suppressed circulation of LH, FSH and estradiol, but not of progesterone. Oral exposure of adult rats to 40 and 81 mg Pb/kg bw/day provoked reduced serum prolactin and LH levels, but did not induce changes in serum testosterone, 17β-estradiol, FSH, LH, prolactin, TSH and thyroid hormones, nor in the histopathology of the gonads and the thyroid gland.

Cadmium

Several studies have shown that cadmium affects hypothalamic-pituitary axis. Acute Cd exposure (5 or 7.5 mg Cd / kg.b.wt s.c.) in rats given in diestrous caused temporary

anovulation, which was preventable by simultaneous luteinizing hormone releasing hormone (LHRH) stimulation (Paksy et al., 1989). Also serum LH and FSH levels of proestrous rats were decreased after 24 hours of treatment. In another study acute cadmium administration decreased plasma LH levels, which was increased after 14 days, thus confirming time dependent effect of the metal on hypothalamic-pituitary axis (Lafuente et al., 1997). Administration of higher dose of the metal to female rats showed decrease in plasma levels of LH and FSH (Paksy et al., 1989; Varga and Paksy, 1991). The route of administration seemed to play a role on cadmium effects on pituitary secretion. Alternately subcutaneous cadmium exposure for 1 month (0.5 mg/kg bosy wt. every 8 days alternating with a dose of 1.0 mg/kg body wt. every 8 days) to adult male rats did not modify plasma gonadotropin levels (Lafuenete and Esquifino, 1999). Hence cadmium administered orally during adulthood seemed to dissociate the regulatory mechanism of gonadotropin secretion. The inhibitory effect of the divalent metals can be due to their direct action at the gonadotrophs and thus inhibiting their secretion. Cooper et al. 1987 showed that in vitro exposure of low concentration of Cd (50 μM) could exert a clear effect on pituitary hormone secretion.

Effect on gonads

Lead

Effects of lead on sperm counts and on the testicles in male rats and on estrous cycles in female rats have been observed at blood Pb levels above 30 μg/100 ml (Chowdhury et al., 1984). Female rats orally exposed to lead acetate for 30 days had irregular estrous cycles at doses of 0.014 mg Pb/kg bw/day (blood Pb 30 μg/dl) and higher. At a dose of 0.26-mg Pb/kg bw/day (blood Pb level 53 μg/dl) these females exhibited follicular cysts with

reduction in the number of corpora lutea in the ovary. Their male counterparts had testicular damage at the higher level (30 ug/dl) and increased prostatic weight at the lower level (19 µg/dl). Shorter exposure to higher doses (1.3 or 5 mg Pb/kg bw/day) leading to blood Pb levels of 70 µg/dl also resulted in longer and more variable menstrual cycles and shorter menstrual flow, but did not prevent ovulation. Lifetime exposure to 0.6% w/v lead acetate in drinking water for gestational day 5, Ronis et al., 1996 have demonstrated disrupted estrous cycling and reduced male secondary sex organ weights in adult offspring accompanied by significant suppression of plasma LH, testosterone and estradiol. Gonadal dysfunction in men, including decreased sperm counts, has been associated with blood lead levels of 40-50 µg/dl (Lancranjan, 1975; Cullen et al., 1984). In male rats treated during three months with 64 mg Pb/kg bw/day (blood Pb level 12 µg/dl) spermatogenesis was adversely affected, and relative testis weights were lower.

Lifetime study of male monkeys orally exposed to doses of lead provoking blood Pb levels of 6-20 µg/dl and 22-141 µg/dl showed changes in sperm chromatin structure and disruption of the general architecture of the seminiferous epithelium (Cullen et al., 1993). This effect was observed after lifetime exposure and after exposure during infancy only. The control group and the group only exposed after infancy did not exhibit this effect. Blood Pb levels of the lifetime and post-infancy exposed groups were 35 µg/dl. while the infancy-exposed and control groups had less than 1.0 µg/dl. In both studies many other reproductive parameters were not affected, e.g. sperm count, viability, mobility and morphology. circulating sexual hormones and absolute testis weights.

Wiebe et al.. 1982 showed that Pb might act in the testis directly (immediate effect) by suppressing enzyme activities. and indirectly (long term effect) by reducing gonadotropin-

receptor binding and the resultant cyclic AMP production. Acute exposure of a mouse leydig tumor cell line to lead resulted in inhibition of hCG stimulated progesterone production and expression of steroidogenic acute regulatory protein (staR) (Huang et al., 1997).

Cadmium

At lower doses in rats, absence of spermiation (6 mg Cd/kg bw) and depression in both sperm and steroid production (0.18 to 0.34 mg Cd/kg bw) without any morphological changes (Hew et al., 1993; Laskey and Phelps, 1991). Male rats receiving 10 mg of Cd/Kg bw orally for 6 weeks daily had significant decrease in sperm production with a decrease in testosterone level and no change in FSH and LH levels (Zenick et al. 1982). Cadmium accumulates in theca layer of the ovary in golden hamsters as well as in non-luteal ovarian tissue of pseudopregnant rats (Denker, 1975). Female rats exposed to cadmium (0.04 to 40 mg Cd/Kg/ day) for 14 weeks, 5 days per week caused an increase in mean duration of estrous cycle, by lengthening diestrous stage, which was more significant in the 40 mg Cd/Kg group (Baranski and Sitarek, 1987). In mature cycling animals ovarian hemorrhagic necrosis was seen within 24 hour after administration of 1 to 3 mg Cd/ kg bw in hamsters, mice and in some strains of rats (Rehm and Waakles, 1988). A single dose of Cd administered in diestrous or proestrous (3.0-7.5 mg/kg body wt. subcutaneous) caused a reduction in serum progesterone concentrations within 20-48 hours after exposure (Paksy et al., 1992). Cadmium given to rats at diestrous stage as a single subcutaneous injection has been shown to inhibit ovulation by lowering the proestrous LH levels (Paksy et al., 1989).

Effect on gonadal hormone metabolism

Liver plays an important role in hormone homeostasis. The induction–alteration of specific steroid-metabolizing enzymes by xenobiotics disrupt hormone homeostasis in the circulating plasma by altering the inactivation, and ultimately the removal, of hormones from the circulation. As the population is exposed to a mixture of compounds that might act at many points in the steroid synthesis–degradation pathway during development and throughout the maturational process. In mice, endosulfan, a common organochlorine pesticide in widespread use today, caused an increase in total testosterone hydroxyl metabolite formation in females. Chao and Chung, 1982 demonstrated that sexual differences in hepatic microsomal drug and steroid metabolism in adult rats are imprinted by androgenic steroids during the neonatal period. Furthermore, alterations in hepatic enzyme activities such as oxido-reductase and glucuronyltransferase are biomarkers for xenobiotic exposure as well as a mechanism to explain altered steroid concentrations in the plasma. However, only a few studies are reported on the effect of heavy metals such as lead and cadmium on hepatic steroid metabolism. Exposure to organic lead is known to affect the metabolism of steroid hormones in humans and mice (Murashow, 1966; Neshkow, 1971; Odenbro et al., 1982). Since the estradiol catabolizing enzymes have sulfhydryl groups and both lead and cadmium are known to bind these sulfhydryl groups, the possibility that changes in the breakdown of these hormones in liver may alter reproductive capacity in man and other animals exposed to these metals can not be excluded.

compete for metal ion transport systems. Similar increase in tissue metal concentration in dams has been observed for cadmium during multiple rounds of gestation/lactation in mice. Increased blood Pb is accompanied by decreased calcium levels during pregnancy has been reported in a prospective study of Swedish women living near a lead smelter (Lagerkvist et al., 1996).

Developmental effects that have been observed in humans following exposure to low levels of lead include reduced birth weight, reduced gestational age and neurobehavioral deficits or delays. Maternal blood Pb levels at which effects were found ranged from ±2.5 to <30 μg/dL. Maternal lead stores readily cross the placenta, placing the fetus at risk.

Pre-natal exposure seems to be more predictive of neurobehavioral lead-related problems than post-natal exposure. Less is know about reproductive effects of chronic exposure to low levels. Lead exposure during pregnancy and the neonatal period may result in delayed sexual maturity, decreased sperm count, loss of masculine sex behaviour, irregular estrous cycling, reduced numbers of corpora lutea, decreased volume of the sexually dimorphic nucleus of the hypothalamus and irregular pulsatile release patterns of gonadotropins in adult offspring. Oral exposure of pregnant and lactating rats and their offspring to doses 3.5-mg Pb/kg bw/day caused delayed vaginal opening in females. The NOAEL for this effect was 0.7 mg Pb/kg bw/day. Similar effects were reported for dams exposed prior to breeding. A recent study has shown that lead exposure during gestation and lactation (12 mg/ml from 30 days prior to breeding until their pups weaned at 21 days) delayed the timing of puberty and this delay was associated with suppressed serum levels of insulin like growth factor-1 (IGF-1), luteinizing hormone and estradiol (Dearth et al., 2002). Also

liver IGF-1 mRNA was not affected suggesting that lead altered translation and/or secretion of IGF-1.

Cadmium

Studies on oral cadmium exposure have not provided evidence of teratogenic effects at dose levels below those that were toxic to maternal animals. Fetotoxic and embryotoxic effects were also observed only at toxic dose levels. Cadmium is known to accumulate in placenta and cause less toxic effects to fetus. Cd is accumulated in placenta with occupational exposed women and smokers (Milnerowicz et al., 2000; Boadi et al., 1992). Cadmium exposure during gestation causes morphological changes of placenta (Hazelhoff Roelfzema et al., 1988). Parenteral Cd administration (0.3 to 3.0 mg Cd/kg bw) between 7 to 16 days of gestation in rodents induces species strain specific teratogenic effects, depending on the dose (Samawarickrama and Webb, 1981; Chernoff, 1973). Sorell and Graziano, 1990 showed that rats when exposed to Cd (0-100 ppm) through water from gestation day 6 to 20 had dose dependent accumulation of Cd and higher doses (50, 100 ppm) showed a decrease in fetal and maternal weights. Cadmium treatment through day 8 to 10 during gestation caused an increase in fetal deaths, increased resorptions and decreased placental weight (Nayak et al., 1989). Cadmium administration (3 or 5 mg/ kg.b.wt S.C. as a single dose) interfered with steroidogenesis in early pregnancy, ovarian estradiol being affected the most (Piasek et al., 2002).

Baranski. 1984 showed that CdCl2 administered during mating and gestation periods at doses of 0.04 to 40 mg Cd / day caused a change in behavioral activity in litters born to Cd treated mothers. Cadmium exposure to female dams (50 ppm) caused a decrease in Zn, Cu, Fe status of the fetus as well as decreased Cu content in placenta, fetal intestine, brain and

kidney (Sowa and Steibert, 1985). Decrease in nuclear and cytoplasmic Zn content in subcellular fraction of fetal liver as well as a decrease in serum Zn, Fe and ceruloplasmin levels were observed in the above study. Cd exposure during gestation (GD 12) inhibits Zn transport system (Samarawickrama and Webb, 1979; Webb and Samarawickrama, 1981) with decreasing rate of DNA synthesis of the fetuses. Neurological effects in rat pups were detected following gestational exposure to 0.4 or 4 mg Cd/kg (Baranski et al., 1986). Decreased fetal weight and minor neurobehavioral alterations in pups have been reported for rats exposed to cadmium oxide (0.16 mg/m3) or cadmium sulfate (about 3 mg/m3) during gestation (ATSDR, 1989).

Biochemical aspects of metal toxicity

Both lead and cadmium are sulfhydryl reactive metals. The sulfhydryl reactive metals have three major properties, which mechanistically explain how they elicit a majority of their toxic effects. First they are transition metals that promote hydrogen peroxide and enhance the subsequent iron and copper induced production of lipid peroxides and the highly reactive hydroxyl radical. Lipid peroxides alter membrane structure and are highly disruptive of mitochondrial function. The pro oxidant properties of the metals are exaculated by their inhibitory effect on antioxidant processes. Lead and cadmium have high affinities for glutathione (GSH), which is the primary intracellular antioxidant and conjugating agent (Quig, 1998). Importantly a single atom of lead or cadmium can bind to and cause the irreversible excretion of up to two GSH tripeptides. The metal GSH conjugation process can deplete the cellular GSH and thus decrease antioxidant capacity. These metal induced depletion of intracellular GSH and increased levels of malonaldehyde in brain and liver have been demonstrated in animal models (Bagchi et al., 1996; Shibasaki

et al., 1996; Karmakar et al., 1998; Nigam et al., 1999). They not only directly remove GSH from the cell but also inhibit the activation of two key enzymes involved in GSH metabolism; GSH synthase and GSH reductase (Daggeett, 1998; Hsu e al., 1998; Adonylo and Otaza, 1999). Both lead and cadmium inhibit the activation of the free radical quenching enzymes catalase, SOD and GSHPx (Howard, 1974; El-Maraghy et al., 2001). The inhibition of GSH peroxidase has been attributed to the formation of a selenide complex. Se is an integral component of GSHPx. The selenium concentration is then insufficient to maintain both the optimal glutathione peroxidase activity and the detoxification of the metal.. Decreased activities of SOD and GSHPx may increase their susceptibility to oxidative injury. Both lead and cadmium can readily displace Zn and Cu, which are cofactors for SOD causing a decrease in the enzyme activity

Phagocytic cells may be another important source of reactive oxygen species in response to metal ions. Furthermore, various studies have suggested that the ability to generate reactive oxygen species by redox cycling quinones and related compounds may require metal ions. Recent studies have suggested that metal ions may enhance the production of tumor necrosis factor-α (TNF-α) and activate protein kinase C, as well as induce the production of stress proteins. Thus, some mechanisms associated with the toxicities of metal ions are very similar to the effects produced by many organic xenobiotics.

Mechanism of lead and cadmium induced effects

The complexity of effects of either Pb or Cd, alone is of interest. the patterns of toxicity of the two metals are similar in some ways and different in others. Perhaps the most significant molecular-level similarity of pathological action for Pb and Cd is their ability to displace and replace special molecules such as Zn and Ca that have pivotal controlling

functions in the body. This is probably also a key demonstration of the differences between the two toxic metals, since there is strong evidence that Pb particularly replaces and disrupts Ca function (Peraza et al., 1998; Bressler et al., 1999), it also affects Zn, and Fe (Peraza et al., 1998), while there is equally strong evidence that Cd particularly replaces/displaces Zn (Peraza et al., 1998; Thiesen and Bach, 1991; Sarkar, 1995), it also affects Ca, as well as Fe and Cu (Peraza et al., 1998).

This ability of toxic metals to be moved into a functional position intended for another similar molecule can have far-reaching effects, especially if the toxic metal rises to an unprecedented concentration. For example, disruptions in either Zn function or Ca function can be expected to have major effects in transcription-controlled signalling and developmental growth involving multiple receptor/ligand systems. This can include the long-term change of response thresholds from chronic low-level exposures occurring during developmental windows.

Lead disruptions of Calcium (and Zinc)

Lead has also been shown to interfere with calcium metabolism, both directly and by interfering with the haem-mediated generation of the vitamin D precursor 1,25-dihydroxycholecalciferol. A significant decrease in the level of circulating 1,25-dihydroxycholecalciferol has been demonstrated in children whose blood lead levels were in the range 12-120 μg/dl, with no evidence of a threshold (Rosen et al., 1980). Tissue lead content is increased in calcium-deficient persons, a fact that assumes great importance in the light of the increased sensitivity to lead exposure that could result from the calcium-deficient status of pregnant women. The interactions between calcium and lead were responsible for a significant portion of the variance in the scores on general intelligence

ratings, and that calcium influenced the deleterious effect of lead. The regulatory enzyme brain protein, kinase C, is stimulated *in vitro* by picomolar lead concentrations (an effect similar to that produced by micromolar calcium concentrations), levels that could be expected from environmental exposure (Markovac and Goldstein, 1988).

However, full pathways of Pb-Ca toxicity mechanisms are still not worked out, presumably because they involve complex Ca second-messenger receptor and transcription modulations (Bressler et al., 1999) that can involve cytokine cascades (Goebel et al., 1999). Since a major target appears to be protein kinase C (PKC) (Bressler et al., 1999), these modulations can occur in all cell types and potentially will have multiple results; PKC is reportedly involved in multiple processes including neurotransmitter synthesis, receptor interactions, ion channel conductances, and dendritic branching (Bressler et al., 1999). Two types of cells that are already well known to be pathologically affected by environmental Pb levels, at least partially via Pb-PKC interactions, are brain (Bressler et al., 1999) and bone (Pounds et al., 1991). More recently a possibly important effect of Pb on the Th1/Th2 balance of gut cytokines has been shown and suggested to be a result of PKC interference (Goebel et al., 1999). Pb also interferes with calmodulin and in Ca2+ pumps (Peraza et al., 1998; Bressler et al., 1999).

A long-recognized Pb effect is interference early in the heme synthetic pathway; ALAD (aminolevulinic acid dehydratase), which decreases linearly with Pb concentration (Marks, 1985) and the resulting build-up of aminolevulinic acid has been used as an indicator of Pb exposure. ALAD is reported to be activated by Zn and inhibited by Pb. This is an important pathway and is required for erythrocyte hemoglobin and liver P-450, among other products (Marks, 1985).

Another Pb interaction of interest that may involve Zn is Pb modulation of NMDA receptors in the brain; particularly the hippocampus (Bressleret al., 1999). Such modulation of NMDARs by Pb, which may also involve PKC (Savolainen et al., 1998). has been suggested as being a key part of the disruption of activity-dependent learning induced by Pb in postnatal development (Johnston and Goldstein, 1998), and may be the main mechanism responsible for the measured IQ loss in children at ambient Pb levels. Pb by impairing Ca^{2+} influx through NMDA (and by impairing PKC and Zn^{2+}) might have similar consequences; including changes in sensitivity (threshold), which may have relevance for CR models. Second, a modulation of certain subunits of NMDARs by Pb has been clearly shown to occur within a short developmental window, and this is suggested to be relevant to learning and memory deficits in children (Nihei et al., 1999).

Cadmium disruptions of Calcium (and Zinc)

Cadmium has been shown to interfere at various levels of cellular Ca signalling. First, with regard to cell surface effects, Cd concentrations in the low micromolar range provoke the formation of inositol phosphates in human skin fibroblasts, causing a large, but short-lasting, mobilization of intracellular free Ca. Secondly, with regard to plasma membrane Ca channels. Cd is a well known blocker of Ca influx into cells. Cd inhibits receptor-operated Ca channels in hepatocytes as well as voltage- operated Ca channels in neuronal cells (Hinkle et al., 1987). However, the latter type of channel is the predominant means by which Cd ions enter mammalian cells. Thirdly, once the heavy- metal ion has reached the cytosol, it interferes with all known active Ca transport systems. Cadmium was shown to be able to substitute for Ca in the activation of calmodulin (Perrino and Chou, 1989). In addition to interacting with -SH groups, cadmium also binds to the calcium-binding site of

the Ca-ATPase (Verbost et al., 1989). This effect might be attributable, at partially, to its similarity in charge and ionic radius to Ca. The interaction between Cd and Ca in bone, intestine, and kidney may result in the disorder of bone metabolism. Ca absorption is decreased by competition with Cd in the intestine, and more Ca is released from maternal bone and transferred to neonate by lactation. In the intestine, Cd uptake competes with Ca uptake. Cd causes a marked decrease in bone density compared to the normal decrease in bone mineral density during lactation.

Many additional macro-level interactions between Cd-Zn, including effects of Zn on Cd absorption and end-organ toxicity, have been shown (Peraza et al., 1998). There is evidence that a Cd-Zn interaction is the basis for pathological effects of maternal smoking on the fetus (Kuhnert et al., 1987)

Further, in the light of the societal prevalence of Zn deficiency, the interactions of Zn primary and secondary deficiencies with Cd or other replacing metals may have long-lasting effects, via transcription-level mechanisms. For instance, if Cd were available at appreciable levels during early Zn deficiency, such as the child having a smoking parent, increased receptor and threshold modulations might occur; as through the Zinc containing neurons (ZcNs) and their interactions with activity-dependent neurotrophic factors (NtF)-based learning (Kuhnert et al., 1987).

Interactions of metals with Zinc Finger Proteins (ZFP)

The Zinc finger is a major structural motif involved in protein-nucleic acid interactions and is present in the largest super family of transcription factors. Zinc ions coordinate this finger like structure through bonds created with cysteine (Cys) and histidine (His) residues. There are codes that may impart selectivity to the action of xenobiotic metals on ZFP. One

is the composition and arrangement of the Cys and His residues, the number of fingers in each protein, and the amino acids that constitute the immediate environment (sphere) of the finger. Another code may lie in the variety of GC box response elements that are recognized by the finger. The shape and composition of the finger will dictate the access and affinity of binding to xenobiotic metals. The DNA consensus sequence will determine recognition and DNA binding of such metalloprotein complexes. Thus metals can interact with the zinc moiety but the consequences of such an interaction will depend on favorable dynamic equilibrium, access to the zinc site and the ability of the cell to repair or counter any adverse effects (Fig. 9).

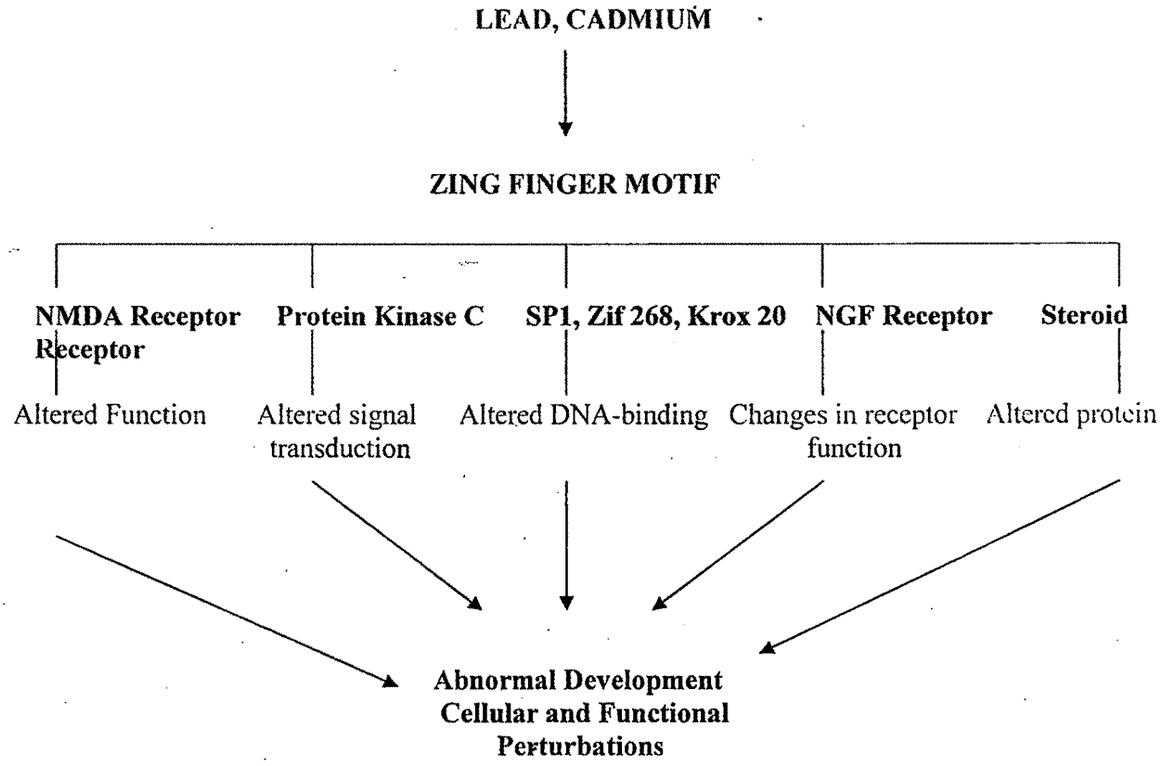
Lead

Metals such as lead interfere with the DNA binding properties of transcription factors, Sp-1 and Egr-1, both *in vivo* and *in vitro* (Zawia et al., 2000). Lead could also directly interfere with the DNA binding of a recombinant human Sp 1 protein. The effects of lead on the DNA binding of the zinc finger protein transcription factor IIIA (TFIIIA) have been demonstrated (Hans et al., 1999). The action of lead on Sp1, Egr-1 and TFIIIA suggests that it can also target other cellular proteins that contain the zinc finger motif and reveals this protein domain as a potential mediator for lead induced alterations in protein function.

Cadmium

It was also found that the zinc finger motif accommodates metals such as cadmium. Cd has been shown to change the binding characteristics of the SP1 transcription factor Zn-finger to DNA (Thiesen and Bach, 1991). Cd can replace Zn in the ER finger and still bind functionally to DNA. However, the cooperativity values for Cd is different from those of the normal dimer (Sarkar, 1995). A change in this cooperativity can result in "recognition

Fig 9: Metals and Zinc Finger Proteins



of a sequence that the protein would not normally bind". This has been suggested as possibly leading to differences in transcription, as well as DNA redox damage from having the replacement metals in that position (Sarkar, 1995)

Direct and indirect genotoxic effects

Lead

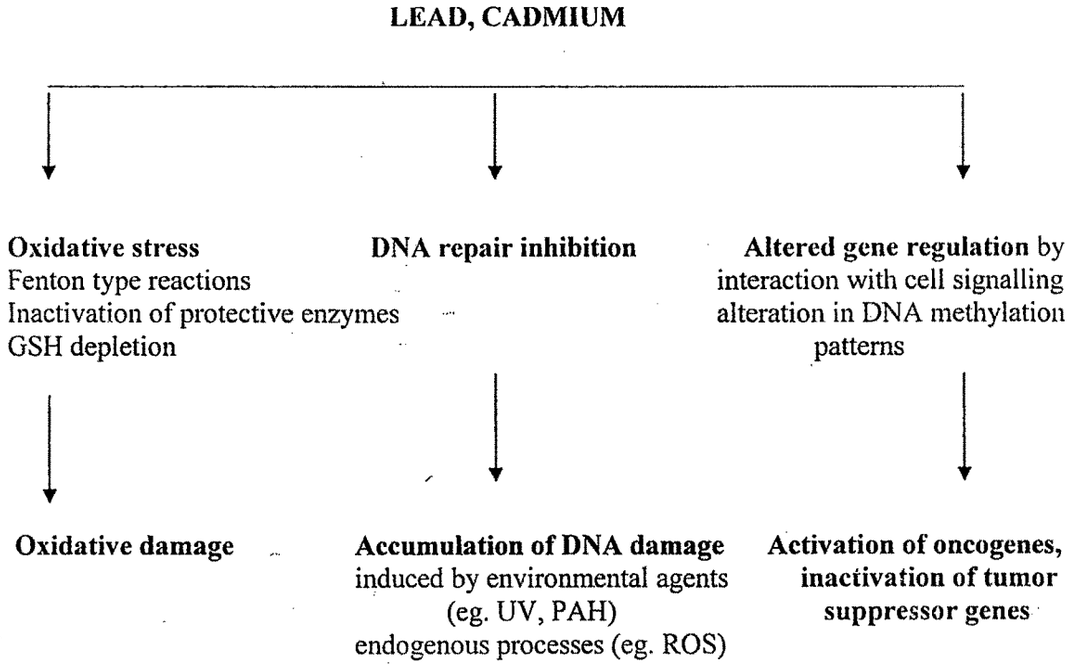
The direct genotoxic effects potential of lead in mammalian cells is weak and mainly restricted to toxic doses. In V79 Chinese hamster cells, both soluble and insoluble compounds of lead have been shown to be weakly mutagenic at the *hprt* locus after 5 days of incubation, additionally the number of cell transformations in Syrian hamster embryo cells was enhanced after treatment with lead acetate (Zelikoff et al., 1988). This weak mutagenic potential has been confirmed recently, where lead acetate was mutagenic at a dose (400 uM) at the E. *coli gpt* locus transfected to V79 cells (Roy and Rossman, 1992). In V79 cells neither lead acetate (0.5-3 uM) ((Zelikoff et al., 1988; Hartwig et al., 1990) nor lead sulfide (500-2000 uM) (Zelikoff et al., 1988)) induced a significant number of sister chromatid exchanges. Furthermore, no DNA strand breaks or DNA-protein cross-links could be detected by alkaline elution or nucleoid sedimentation. In contrast at toxic doses (400 uM) lead acetate and lead nitrate induced DNA strand breaks determined by nick translation. More pronounced comutagenic activities in combination with other DNA damaging agents. Lead acetate enhanced the frequencies of ultraviolet (UV) induced mutations at the *hprt* locus as well as sister chromatid exchanges. These effects seems to be due to an interference with DNA repair processes, since lead ions caused an accumulation of DNA strand breaks after UV irradiation, as shown in HeLa cells. Since the repair of UV induced DNA damage is mediated by the nucleotide excision repair pathway,

which is characterized by the transient occurrence of DNA strand breaks as a result of incisions at the sites of damage, an accumulation of breaks points toward an inhibition of the polymerization or ligation step by lead. A decreased fidelity of the DNA polymerase from avian myeloblastosis virus (AMV) in the presence of lead chloride has been shown (Sirover and Loeb, 1976). Similarly DNA and RNA synthesis was reduced in intact nuclei from HeLa cells after incubation with 80 and 150 uM lead acetate. No inhibition of DNA or RNA synthesis however was found in intact HeLa cells after exposure to 500 uM lead acetate for upto 18 hr, indicating lack of bioavilability of lead ions (Frenkel and Middleton. 1987) (Fig. 10).

Cadmium

In V79 Chinese hamster cells, $CdCl_2$ induced DNA single strand breaks, DNA–protein cross-links (20-200 uM) (Ochi and Ohsawa, 1983) and chromosomal aberrations (10-50 uM) (Ochi et al., 1984). It enhanced the number of mutations at the thymidine kinase locus in mouse lymphoma L51784/TK+/- cells and at the *hprt* locus in the V79 cells. Concerning the generation of DNA single strand breaks and chromosomal aberrations the involvement of reactive oxygen species has been shown (Ochi and Ohsawa, 1983; Synder, 1988) which might be mediated by a decrease in intracellular glutathione induced by Cd at similar concentrations (Ochi et al., 1984). In addition to the direct genotoxic effects described, one other line of evidence suggests that enhancement of genotoxicity of other DNA damaging agents by cadmium, is possibly by interfering with DNA repair processes involved in the removal of DNA damage by alkylating agents or UVC irradiation. In bacterial test systems a comutagenic effect of cadmium in combination with methyl nitrosourea (MNU) was observed in *S. typhimurium* (Mandel and Ryser, 1984) and confirmed in *E. coli* (Takahashi

Fig 10: Metal induced DNA damage



et al., 1988). The latter effect was due to the inactivation of the O6-methulguanine DNA methyl transferase (MGTase) (Takahashi et al., 1992). The effect of cadmium on this protein was attributed to inhibition of its transcriptional activity possibly by binding to critical methyl group acceptor sites. When the MGTase was isolated from E. coli, rat liver or human cells, it was inactivated by cadmium (Bhattacharya et al., 1988). However no data concerning the interaction of cadmium with alkylating agents in mammalian cells are currently available.

Also enhancement of UV induced mutagenicity by $CdCl_2$ in V79 cells as well as an increase in UV induced cytotoxicity by nontoxic concentrations of $CdCl_2$ in various cell lines including V79, CHO, HeLa and human fibroblasts were observed (Hartwig and Beyersmann, 1989). Concerning isolated enzymes involved in DNA replication and repair, an inhibition of DNA polymerase beta at low concentrations of cadmium acetate was observed (Popenoe and Schmaeler, 1979) as well as a decrease in the fidelity of DNA polymerization in the presence of cadmium (Sirover and Loeb, 1976).

Adaptive mechanism

Several studies have suggested that body makes important adaptive changes in response to exposure to heavy metals. The importance of GSH metabolism in response to lead and cadmium are well documented. It has been hypothesized that GSH may provide a first line of defense against cadmium toxicity in the absence of appreciable metallothionein (MT) concentration (Quig, 1998). MTs are a fascinating group of low molecular weight intracellular proteins that serve as a storage depot for Co and Zn and scavenge sulfhydryl reactive metals that enter the cell. MT has an unusual amino acid composition in that it has no aromatic amino acids and one-third of its residues are cysteines. These cysteine residues

bind and store metal ions. Pharmacodynamic tolerance occurs via high-affinity sequestration of the metal within the cell. As a result most of the Cd in cells is bound to MT in the cytosol, with a concomitant reduction of the Cd available to bind/damage critical organelles. Thus, induction of MT is an important adaptive mechanism preventing metal toxicity in animals as well as in humans. Newborn animals have high concentrations of MT in their livers; thus they are resistant to Cd-induced lethality and hepatotoxicity. In essence induction of MT has been proposed as an important adaptive mechanism in response to environmental stimuli. Induction of MT protects against metal toxicity, acts as a free radical scavenger protecting against oxidative damage, and protects against toxicity. Lead also binds metallothionein, but does not appear to displace cadmium or zinc. Metallothionein is apparently not induced by lead, although metallothionein sequesters lead in the cell. Another lead-binding protein is acidic, carboxyl-rich protein found in the kidney and brain (ATSDR 1999).

According to various environmental protection agencies like ATSDR, WHO, SCOEL various pollutants and toxicants are rapidly increasing in the environment and becoming a global health problem. Bioaccumulation of heavy metals including lead and cadmium is also cause of concern. In view of this, various epidemiological as well a s animal studies has been performed targeting their effects in different organ systems including reproductive system. Toxicants and pollutants, which mimic hormones, termed as endocrine disrupters. Recently scientists have targeted their research to understand the role of endocrine disruptors, as endocrine is one of the important systems, which regulate the homeostasis of various body functions including reproduction.

Aim and objectives of the present study

Reproduction is one of the major aspects of life and is under the influence of neuroendocrine regulators. It becomes clear from the review that both lead and cadmium can affect the activity of the hypothalamic-pituitary-gonadal axis by acting at the hypothalamus, the pituitary, the ovary and the steroid metabolizing tissue, liver. Since gonadotropins and sex steroids control many reproductive functions, the possibility that changes in the synthesis or breakdown of these hormones may alter reproductive capacity in man and other animals exposed to these heavy metals cannot be excluded. **Hence in the present study an attempt has been made to understand the role of lead and cadmium as endocrine disruptors.**

Despite the fact that toxic waste disposal sites and other exposure vectors common to lead and cadmium often involve both metals, most research on these two metals has dealt with each in isolation; that is, most investigations are designed to examine only lead or cadmium. On the other hand, populations in real life always have simultaneous multiple exposures indicating the need for experimental work with combination of substances. Based on this the earlier dose dependent study performed in our laboratory with low level isolated and combined exposure of lead and cadmium have shown that dose as low as 0.1 mg/kg body weight/day for 30 days can also affect δ-Aminolevulinic acid dehydratase (marker of lead toxicity) activity, differentially in isolated and combined state (Gupta et al., 1994). **In light of this and the various deleterious effects of these metals on reproduction and endocrine function as cited above, it was worthwhile to study the simultaneous exposure of lead and cadmium on hypothalamus-pituitary axis function in relation with female reproductive system. Efforts have also been made to understand the mechanism of interaction of lead and cadmium.**

Objectives of the study

I. Dose dependent study on the effects of lead and cadmium in isolation and combination on hepatic, pituitary and hypothalamic steroid metabolism in non pregnant rats.

II. To study the effects of lead and cadmium in isolation and combination in non pregnant rats on hypothalamic-pituitary axis function.

III. To study the effects of lead and cadmium in isolation and combination in pregnant rats on

(a) Reproductive performance

(b) Hepatic estradiol metabolism

(c) Hypothalamic-pituitary axis function

IV. The mechanism of action of lead and cadmium either alone or in combination on liver and hypothalamic-pituitary axis.