Endocrinology of Reproduction

Endocrinology: is the branch of biological science that deals with the study of hormones and their receptors

Hormone: The classic definition of a hormone is a physiologic, organic, chemical substance produced by certain specialized cells which passes into the circulatory system for transport in order to stimulate or inhibit the functional activity of the target organ of tissue. The hormones controlling the reproductive processes are derived primarily from the areas of the hypothalamus, pituitary, gonads, placenta and uterus.

Techniques in endocrinology

Ablation. The surgical removal of the endocrine gland will lead to a deficiency syndrome of the hormone produced by the gland. One of the oldest and most common examples of ablation of an endocrine gland is castration of male domestic animals. This removes androgen from the male animal, which results in a change in physical characteristics

Replacement therapy. The deficiency syndrome caused by ablation may be overcome by implantation of the gland back into the animal or by injection of crude extracts from the removed gland

Classification and properties of hormone

-The hormone of reproduction are divided into two groups based on type and action

1-the primary hormones of reproduction: these hormones are directly involved in the various aspects of reproduction such as spermatogenesis, ovulation, sexual behavior, fertilization, implantation, maintenance of gestation, parturition, lactation and maternal behavior.

2-metabolic hormones that influence reproduction: these metabolic hormones are necessary for the general well-being and metabolic state of the animal and permit reproduction to occur. These metabolic hormones maintain the state of animal and thereby permit the full effect of the primary hormones of reproduction.

The hormones of reproduction are divided into three categories according to their chemical Structure:

1-Proteins : molecular weight range 300-70000 daltons and can administered by injection only

2- steroids : molecular weight approximately 300-400 daltons and can administration by injection and orally

3- fatty acids : molecular weight 400 daltons and can administration by injection only

Hormones of Reproduction

(a) Hypothalamus (Releasing hormones)

1-GnRH (peptide hormone 10 amino acids)

Function

Stimulates release of FSH, LH

2- TRH (peptide hormone 3 amino acids)

Function

Stimulates release of TSH and prolactin

3- prolactin inhibiting factor (PIF)

Function

Inhibits prolactin release

4- Oxytocin (stored in posterior pituitary also produced in ovary)

Function

Stimulates uterine contractions, parturition, sperm and egg transport, milk ejection possible luteolytic action

5- Corticotrophin releasing hormone (CRH) peptide hormone 41 amino acid

Function

To release ACTH

(b) Anterior pituitary

1- follicle stimulating hormone (FSH) :glycoprotein hormone

Function

Stimulates follicular growth , spermatogenesis, estrogen secretion

2- Luteinizing hormone (LH) : glycoprotein hormone

Function

Stimulates ovulation, corpus luteum function; stimulates secretion of progesterone, estrogen and androgen

3- Prolactin (protein hormone 198 amino acid)

Function

Promotes lactation; stimulates corpus luteum function and progesterone secretion in some species; promotes maternal behavior, promotes tissue and bone growth.

(c) Placenta

1- Human chorionic gonadotrophin (hCG): glycoprotein hormone.

Function

Demonstrates LH activity, maintains CL of pregnancy in primate

2- Pregnant mare serum gonadotrophin (PMSG): glycoprotein hormone

Function

Demonstrates FSH activity; stimulates formation of accessory CL in mare

3- Placental lactogen (protein hormone).

Function

Regulation of maternal nutrients to fetus

4- Protein B:

Function: Unknown

(d) Gonads

1- Estrogen (steroid hormone)

Function

Promotes female sex behavior, stimulates secondary sex characteristics, growth of reproductive tract, uterine contractions, mammary duct growth, controls gonadotrophin release, stimulates calcium uptake in bones, has anabolic effects

2- **Progesterone** (steroid hormone)

Function

Acts synergistically with estrogen in promoting estrous behavior and preparing reproductive tract for implantation, stimulates endometrial secretion, maintains pregnancy, stimulates mammary alveolar growth, and controls gonadotrophin secretion

3- **Androgen** (steroid hormone)

Function

Develops and maintains accessory sex glands, stimulates secondary sexual characteristics, sexual behavior, spermatogenesis, and has anabolic effects

(e) Uterus

1- **Inhibin** (protein hormone)

Function

Inhibits FSH release

2- **Relaxin** (poly peptide hormone)

Function

Dilates cervix

3- **PGF2α** (fatty acid)

Function

Causes uterine contractions and is luteolytic

Mechanism of action of protein hormone

Protein and polypeptide hormones regulate cell function by binding to a cell-membrane-specific receptor that controls the activity of the enzyme, **adenylate cyclase**, which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) .

The discovery by Sutherland and Rall (1960) of this cyclic nucleotide led to Sutherland's proposal of second messenger hypothesis.

The first messenger is the hormone, which interacts with the receptor site upon the cell surface of the target organ.

This induces either an increase or decrease in the activity of enzyme adenylate cyclase(a component of the cell membrane), which results in an increase or decrease in the concentration of cAMP (the second messenger within the cell) .

The cAMP acts on an enzyme called a cAMP-dependent protein Kinase, which consists of two dissimilar subunits, a regulatory one and catalytic one.

The cAMP combines with the regulator subunit causing dissociation of the two subunits. When associated with the regulatory subunit, the catalytic subunit is inhibited. When free, the catalytic subunit is active and catalyze the phosphorylation of one or more specific proteins within the cell.

This phosphorylation may be of histone proteins that unmask DNA. The unmasked portion of chromosomal DNA, which contains the genetic information for production of a specific enzyme, allows transcription by RNA polymerase. This result in the synthesis of new mRNA that goes back out in the cytoplasm to produce the new protein that may be an important enzyme in steroid biosynthesis.

The enzyme responsible for the destruction of cAMP is **phosphodiesterase**, which catalyzes the hydrolytic reaction. This enzyme is inhibited by caffeine and theophylline, alkaloids present in coffee and tea. These drugs can prolong the intensity of a hormone, presumably due to increased persistence of cAMP in cells stimulated by the hormone.

Mechanism of action of steroid hormones

Steroid hormones pass through both the cell membrane and cell cytoplasm before binding to receptors in the nucleus. The passage of steroids through the cell membrane and cytoplasm is probably by simple diffusion. The binding of steroid hormone to its receptor start the synthesis of specific mRNA which is translocated to the cytoplasm where it directs synthesis of specific proteins such as actin and myosin, which act on contraction of uterine muscles that important in parturition.

Hypothalamus-pituitary anatomy

The hypothalamus lies at the base of the brain, bordered anteriorly by the optic chiasm, posteriorly by the mammillary bodies, dorsally by the thalamus and ventrally by the sphenoid bone. Its entire size is about 1/300 of the entire brain. The hypothalamus is composed of many bilaterally-paired nuclei such as adenohypophysis(AH), anterior hypothalamic area (AHA),dorsal hypothalamic area (DHA), dorsal medial nucleus(DMN), median eminence (ME), neurohypophysis (NH), mammillary body (MB), optic chiasm (OC), paraventricular nuclei (PVN) and posterior hypothalamic area (PHA) . The medial portion of the hypothalamus, known as the third ventricle of brain, separates most of the paired nuclei.

The pituitary gland lies below the hypothalamus in a bony depression in the sphenoid bone called the ***sella turcica***. In the embryo, the pituitary gland develops from the gut ectoderm of the roof of the mouth and the neural ectoderm of the developing hypothalamus. This double origin is partly carried over into the adult organism wherein the two major divisions are retained as distinct entities, the anterior pituitary and the posterior pituitary gland.

A unique vascular connection exists between the hypothalamus and the anterior pituitary. Arterial blood comes into the pituitary by way of the superior hypophyseal artery and inferior hypophyseal artery. The superior hypophyseal artery forms capillary loops at the median eminence and pars nervosa. From these capillaries, the blood flows into the hypothalamo-hypophyseal portal vessels, which pass down the pituitary stalk and terminate in capillaries in the anterior pituitary.

A portal system begins and ends in capillaries without going through the heart. The hypothalamo-hypophyseal portal system is the vascular pathway that transports hypothalamic hormones to the anterior pituitary.

The inferior hypophyseal artery transports blood to the anterior and posterior pituitary. Not only does blood flow from the hypothalamus to the pituitary, but also part of the venous outflow of the anterior pituitary is by way of a retrograde flow to the hypothalamus. The physiologic importance of these findings is significant because they are associated with a negative feedback regulation of hypothalamus by the pituitary hormones. This type of the feedback has been termed the short- loop feedback.

Hypothalamic Hormone

Oxytocin

Oxytocin and vasopressin are synthesized in the hypothalamus and stored in the neurohypophysis (posterior pituitary) . Oxytocin is found in all mammals. Oxytocin and vasopressin are synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and only stored and released from the neurohypophysis, therefore called neurohypophyseal hormones. Oxytocin and vasopressin are transported in small vesicles enclosed by a membrane. These secretory vesicles flow down the hypothalamic-hypophyseal nerve axons by axoplasmic streaming and are stored at the nerve endings next the capillary beds in the neurohypophysis until their release into the circulation. Oxytocin is also produced in the corpus luteum of ewe, cow, and human. Therefore oxytocin has two site of origin, the ovary and the hypothalamus.

Function of oxytocin

Oxytocin in Greek means rapid birth, thus describing one of its physiologic functions, which is contraction of uterine muscle. Oxytocin also causes increased contraction frequency in the oviduct and may thus be involved in the transport of both female and male gametes in the oviduct. How oxytocin directly affects uterine and oviduct contraction is not known, however estrogen enhances the responsiveness of smooth muscle to oxytocin. In the bird and reptile vasotocin appears to be important in causing contraction of the shell gland and vagina to induce oviposition.

The milk ejection reflex or milk let-down is an example of a neuroendocrine reflex. This is one of the best established functions of oxytocin. The lactating female becomes conditioned to visual and tactile stimuli associated with suckling or milking. This conditioning induces the release of oxytocin into the circulation. Oxytocin then acts on the myoepithelial cells in the mammary gland. The contraction of the myoepithelial cells puts pressure on the alveoli, which displaces milk into the duct system of the mammary gland, resulting in milk letdown.

Exogenous oxytocin has a luteolytic action in the cow and goat also immunized ewes against oxytocin show a persistent corpus luteum. This suggests that ovarian oxytocin may be involved in luteal function by acting on the endometrium of the uterus to induce $PGF2\alpha$ release, which has luteolytic action.

Application

Oxytocin is used to induce female animals to let down milk after parturition if a problem exists and to induce expulsion of retained placentas. It is also used to aid delivery in young animals when the female has been in labor for an extended period.

Luteinizing Hormone Releasing Hormone (LH-RH)

Luteinizing hormone releasing hormone (LH-RH) is a decapeptide (10 amino acids) with molecular weight of 1183 daltons. This hormone induces release of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary. The synthesis of a large number of structural analogs of LH-RH has been important in establishing the structure activity relationship of this hormone. Two basic types of analogs to LH-RH have been synthesized. The antagonistic analogs appear to bind at the receptor site on the pituitary but do not induce the release of LH or FSH and block the action of the natural hormone. Stimulatory analogs have been synthesized that induce release of more LH and FSH than natural LH-RH. The first three amino acids are necessary for activating LH and FSH release. The reason for the increased biologic activity of LH-RH agonists is due to their ability to stay bound to the pituitary receptor longer than the natural hormone and their ability to resist enzyme degradation. The half-life of LH-RH is 7 minutes in the ewe. The short half-life of LH-RH has made its application to the livestock industry difficult. There are three other structurally different naturally occurring LH-RH molecules, two of which are in the chicken and the other in the fish but in frog has the same LH-RH structure as the mammal.

Application of LH-RH

LH-RH is effective in overcoming cystic follicles in cows. In this instance, 100 µg of LH-RH induces the release of substantial amounts of endogenous LH to induce luteinization or rupture of the cystic follicle. A cow with a treated cystic ovary will exhibit estrus 19 to 2 days later and can then be bred.

Other Hypothalamic Hormones

Other hypothalamic hormones that have been isolated and structurally identified are thyrotrophin releasing hormone (TRH) 3 amino acids, somatostatin or growth hormone inhibiting hormone(GH-IH)14 amino acids, growth hormone releasing hormone (GH-RH) 44 amino acids and corticotrophin releasing hormone (CRH) 41 amino acids.

Pituitary Gonadotrophic Hormone

The anterior pituitary gland secretes three gonadotrophic hormones, follicle stimulating hormone, luteinizing hormone and prolactin. These hormones have primary action on the gonads.

Follicle Stimulating Hormone (FSH)

FSH has also been termed follicotrophin. The anterior pituitary gland secretes three glycoprotein hormones. These are FSH, LH and thyroid stimulating hormone (TS H).

These hormones have two non identical subunits termed alpha (α) and beta (β). The alpha subunit is identical within species for FSH, LH and TSH. The molecular weight of each of these 3 glycoprotein hormones is approximately 32000 daltons, with each subunit having a molecular weight of 16000 daltons.

The α and β subunits of any of these hormones by themselves have no biologic activity. If the α subunit of one hormone (LHα) is recombined with the β subunit of another hormone (FSHβ), the molecule regains FSH biologic activity or the activity of the β subunit. If two α subunit or two β subunit are combined, no biologic activity is noted. The α subunit of FSH contain 92 amino acids with carbohydrate side chain at amino acid 52 and 72. The half-life of FSH is approximately 2- 2.5 hours.

Function of FSH

In female animal, FSH stimulates the growth and maturation of graafian follicle in the ovary and is, therefore, the primary factor inducing growth of the ovary. FSH will not cause estrogen secretion from the ovary by itself, but in the presence of LH, it will stimulate estrogen production from either the ovary or testis. In the male, FSH acts on the germinal cells in the seminiferous tubule to enlarge the testis. FSH is also responsible for spermatogenesis up to the secondary spermatocytes after which androgens are responsible for final stages of spermatogenesis.

After menopause the pituitary output of FSH in women increases tremendously due to the lack of steroid output. This increase in FSH output is at such high concentration that it passes through the kidney and goes directly to the urine and is called human menopausal gonadotrophin (hMG). The biologic activity of hMG is increased over FSH from women with active ovaries and is sold as a fertility hormone for women under the trade name of **pergonal**.

Application

Now, FSH is primarily used in the stimulation of follicular development to induce multiple ovulations for embryo transfer.

Luteinizing Hormone (LH)

Luteinizing hormone has also been termed luteotrophin and interstitial cell stimulating hormone (ICSH).

Chemical Nature. Luteinizing hormone is a glycoprotein composed of an α and β subunit with molecular weight of 30000 daltons and a half-life of 30 minutes.

Function of LH

Tonic or basal levels of LH act in conjunction with FSH to induce estrogen secretion from the large graafian follicle. The pre-ovulatory surge of LH is responsible for rupture of follicle wall and ovulation. Evidence indicates that LH is the main luteotrophic substance in domestic animals. Prolactin, however, also plays a role in maintaining the functional of the corpus luteum. The interstitial cells of both the ovary and testis are stimulated by LH. In the male, the interstitial cells (Leydig cells) produce androgens after LH stimulation.

Placental Hormones

Pregnant Mare Serum Gonadotrophin (PMSG)

PMSG is a glycoprotein with α and β subunits similar to LH and FSH but with a higher content of carbohydrates, especially sialic acid. The higher sialic acid content appears to account for the long half-life of several days for PMSG. Thus a single injection of PMSG can have biologic effects at the target gland for more than a week. The molecular weight estimates of PMSG vary, but are in the range of 60000 daltons. This placental gonadotrophin is secreted by endometrial cups in the equine uterus. These structures are formed by specialized trophoblastic cells that invade the maternal endometrium and are thus believed to be primarily of fetal origin. The fetal genotype of PMSG is secreted from the endometrial cups. For example, a mare horse pregnant with a mule fetus will have lower circulating concentrations of PMSG than if she were pregnant with a horse fetus.

Function

The endometrial cups are formed about day 40 of pregnancy and persist until day 85 of pregnancy. The secretion of PMSG stimulates development of follicles on ovary due to the FSH effect of the PMSG. Some of these follicles then ovulate, but most follicles simply form a luteinized follicle due to the LH- like action of the PMSG molecule. These accessory corpora lutea produce progestogens important to the maintenance of pregnancy in the mare. Thus, PMSG is described as having both FSH and LH biologic actions wit the FSH actions being dominant. PMSG is isolated from the blood of pregnant mares and is not found in urine may be due to its large size does not allow it to clear the glomerular capsules of the kidney.

Application

PMSG was one of the first commercially available gonadotrophins and is used to induce superovulation in domestic animals. It is one of the products available for superovulation in embryo transfer of cattle, sheep and pigs. It is ineffective in mare doses feasible for administration.

Human Chorionic Gonadotrophin (hCG)

The chemical structure of hCG has been well defined as a glycoprotein consisting of α and β subunits with molecular weight of 40000 daltons. The α subunit has 92 amino acids and 2 carbohydrate chains. The α subunit of hCG is similar to the α subunits of human, porcine, ovine and bovine LH. The β subunit has 145 amino acids and 5 carbohydrate chains. Human chorionic gonadotrophin is synthesized by the syncytiotrophoblastic cells of the placenta of the pregnant primate and is found in both the blood and urine. It has been detected in the urine as soon as 8 days after conception by sensitive radioimmunoassays. Human chorionic gonadotrophin has both LH and FSH- like actions, but has predominately LH-like biologic actions. It converts the corpus luteum of the menstrual cycle in the human to the corpus luteum of pregnancy.

Application

Since hCG appears early in the human pregnancy, detection of hCG in the urine is the basis of immunological human pregnancy tests presently available on the market. In addition, the LH- like action of hCG has made it the first hormone available for treatment of a cow with cystic ovaries usually requires 5000-10000 IU of hCG.

Gonadal Hormones

Relaxin

Relaxin is a polypeptide hormone consisting of α and β subunits with molecular weight 57000 daltons and has structure similarity to insulin, but they have different biologic actions. Relaxin secreted primarily by the corpus luteum of the ovary during pregnancy. In addition, the placenta and uterus secrete relaxin in some species.

The main biologic action of relaxin is dilation of the uterine, cervix and vagina prior to parturition. It also inhibits uterine contraction and causes increased growth of the mammary gland if given in conjunction with estradiol.

Inhibin

Inhibin, also termed **folliculostatin** is a protein hormone that has been chemically identified. It is produced by the sertoli cells in the male and the granulosa cells in the female. Inhibin can inhibit FSH release from the pituitary without altering LH release and is partially responsible for the differential release of LH and FSH from pituitary.

Gonadal Steroid Hormones

Steroidal hormones are secreted not only by the ovary and testis, but also by the placenta and adrenal cortex. Steroids have a basic or common nucleus called **the cyclopentano-perhydrophenathrene nucleus**. Steroid producing tissues possess the enzymes necessary for synthesis of all steroid hormones. The ability of different cells to secrete specific steroid hormones depends on the quantity of individual enzymes contained in the cell.

Androgens

Testosterone belongs to the class of steroids known as androgens and have 19 carbon atoms. In the male, androgens are produced by the interstitial cells (Leydig cells) of the testes, with a limited amount being produced by the adrenal cortex. The horse is the unique species because the seminiferous tubules and epididymis also produce testosterone in the high levels.

Testosterone is transported in the blood by an alpha globulin designated steroid-binding globulin. Ninety-seven to ninety- nine percent of circulating testosterone is bound. The remaining testosterone is free to enter the target cell where an enzyme in the cytoplasm converts testosterone to dihydrotestosterone, which can proceed to act on the nuclear receptor.

Function

Androgens stimulate the latter stages of spermatogenesis and prolong the life span of epididymal sperm. They promote growth, development and secretory activity of the accessory sex organs of the male such as the prostate, vesicular glands, bulbourethral gland, vas deferens and external genitalia (penis and scrotum).The maintenance of secondary sex characteristic and sexual behavior or libido of the male is controlled by androgens. They induce protein anabolic activity that involves the entire organism (nitrogen retention) and have also been found to increase sebaceous gland development. Androgens have a negative feedback effect on the hypothalamic-pituitary axis in control of LH and FSH release.

Estrogens

Substances of estrogenic activity have been found in both the animal and plant kingdom and have 18 carbon atoms. In the synthesis of this hormone a unique development called the two-cell two- gonadotrophin hypothesis can occur in both the female and male. LH stimulates thecal cells of follicles to secrete testosterone. The testosterone is subsequently aromatized to estradiol in granulosa cells under the influence of FSH stimulation. This so called two-cell gonadotrophin model is similar to that previously demonstrated in testicular tissue in which LH stimulates testosterone production in the Leydig cells and FSH stimulates aromatization of the testosterone to estrogen in the sertoli cells of the seminiferous tubules.

Function

Estrogens, like androgens, are carried by binding protein in the circulation. Of all the steroids, estrogens have the widest range of physiologic function. Estrogen acts on the central nervous system to induce behavioral estrus in the female; however, small amounts of progestogen with estrogen are needed in some species such as the ewe and cow to induce estrus. The first ovulation in the ewe at puberty or the start of breeding season is without estrus because only estrogen is present in circulation, but at the second ovulation, the estrogen from the follicle to ovulate and the progesterone from the waning corpus luteum together induce behavioral estrus in this species. Estrogen act on the uterus to increase the mass of both the endometrium and myometrium. The increased growth is due both to cell hyperplasia and hypertrophy. It also acts on the uterus to increase both amplitude and frequency of contraction by potentiating the effects of oxytocin and prostaglandin F2α. Physical development of female secondary sex characteristics are attributed to estrogen. Estrogen stimulates duct growth and causes the development of the mammary gland. Estrogens have both a negative and positive feedback control through the hypothalamus on LH and FSH release: the negative effect is on the tonic center in the hypothalamus and the positive effect is n the preovulatory center. Non reproductive effects of estrogen include stimulation of calcium uptake and ossification of bone also have a protein anabolic effect lead to increase in weight gain and growth.

Progestogens

Progesterone is most prevalent, naturally occurring progestogen and is secreted by luteal cells of the corpus luteum. This hormone is also secreted by the placenta and adrenal gland. Progesterone has 21 carbon atoms and is transported in blood by a binding globulin much like androgens and estrogens.

Function

Progesterone prepares the uterus for implantation and maintenance of pregnancy by increasing secretory glands in the endometrium and inhibiting the motility of the myometrium. Progesterone acts synergistically with estrogens to induce behavioral estrus in sheep and possibly in cattle. It develops the secretory tissue (alveoli) of the mammary gland. High levels of progesterone inhibit estrus and the ovulatory surge of LH, thus establishing the importance of this hormone regulation of the estrous cycle.

Application

In humans and domestic animals, progestogens are given to prevent abortion in females prone to abortion or miscarriage due to insufficient endogenous progesterone production also is used in synchronization of estrus in cow and ewe.

Uterine Hormones

Prostaglandins

Unlike other humoral agents, prostaglandins are not localized in any particular tissue. Most prostaglandins act locally at the site of their production on a cell-to-cell interaction and therefore do not conform exactly to the classic definition of a hormone. They can also be transported in the blood to act on a target tissue away from the site of production.

Chemistry. Prostaglandins exist in the form of at least six parent compounds and numerous metabolites, which exhibit a wide variety of pharmacologic effects. Prostaglandins are involved in control of blood pressure, lipolysis, gastric secretion, blood clotting and other general physiologic processes including renal and respiratory function. Blood levels of prostaglandins are generally low but are elevated under certain conditions such as parturition. Prostaglandins are rapidly degraded in the blood, and it is only after injection with pharmacologic or high levels of prostaglandin that a sustained physiologic effect is noted. All prostaglandins are 20-carbon unsaturated hydroxy fatty acids with a cyclopentane ring. Arachidonic acid, an essential fatty acid, is the precursor for prostaglandin, mainly prostaglandin F2α (PGF2α) and prostaglandin E2 (PGE2).

Function

Evidence that prostaglandins are involved in ovulation is found in the ewe and cows. Ovulation in the ewe and cow is blocked by the administration of indomethacin, an inhibitor of prostaglandin synthesis. LH release is not affected in these animals so the action and synthesis of prostaglandin is probably at the level of the ovarian follicle involving either or both PGF2α and PGE2.

PGE2 stimulates contraction of the uterus, dilates blood vessels and has no luteolytic action. PGF2α stimulates contraction of the uterus, aids in sperm transport in the male and female, causes constriction of blood vessels and has luteolytic properties in domestic animals.

Application

Prostaglandins, available through veterinarians, are used in timed breeding in cows and mares and in abortion of cattle. The trade names of these compounds are Lutalyse and Estrumate for the cow, and prostin for the mare. Prostaglandin F2α has also been found to aid in treating the infected uterus of dairy cows. Its mechanism of action is possibly twofold: first by regression of the CL, if present, and thus induction of follicle growth and estrogen production, and second by contraction of the uterus both from its action and from the action of estrogen.