

Selected Biochemical Actions of Ovarian Hormones

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This review is concerned with a general discussion of the actions of ovarian hormones, including relaxin, a polypeptide hormone, and estrogens and progestogens, which are two types of steroid hormones. Particular emphasis is placed on describing the sites of action and target organ responses induced by the two steroid hormones. A detailed description of estrogen activity in reproductive tract tissue is given as an example of the intracellular mechanism of action of this steroid hormone.

Ovarian Secretion

Two types of biochemical compounds with hormonal activity are synthesized and secreted by ovarian tissue. One is a polypeptide hormone, originally described some years ago by Hisaw (1), termed relaxin. The second is a class of compounds with steroidal structure which includes androgens, estrogens and progestogens. The ovary possesses the enzymatic capability to synthesize all three types of steroids. Such activities are localized in the luteal and interstitial compartments for androgen and progesterin synthesis (2). Estrogenic steroids appear to arise from thecal and granulosa cell elements of the ovary (3). This discussion of ovarian steroid action within the female will center on estrogens and progestogens, since they are considered to be the primary steroids secreted by the ovary. Although the actions of these steroid hormones are of primary concern, a brief mention of our present knowledge of the action of relaxin will also be made.

Polypeptide Hormones

Relaxin has been described as a simple protein being composed of two polypeptide chains and

having a molecular weight of approximately 6000 daltons (4). The hormonal activity described has been classically detected during pregnancy in plasma and urine extracts. As mentioned earlier, the material is of ovarian origin and recent reports have indicated its localization in corpora lutea (5). Its exact biological function is unclear, but in the human the action of relaxing and dilating cervical and vaginal tissue just prior to and during parturition is the presently accepted notion. In laboratory animals, relaxin has been shown to cause dilation of the cervix in mice and rats (1), a lengthening of the pubic symphysis of mice and guinea pigs (6), and inhibition of uterine contraction in rats and guinea pigs. These effects would suggest a general action on the smooth muscle in reproductive tract tissue. The biochemical mechanism of relaxin action in a target cell is unknown, but recent work indicates the hormone binds with high affinity ($K_a \cong 10^9-10^{10} M^{-1}$) and specificity to a membrane-associated receptor from rat uteri (7). Since this binding is enhanced by estrogens, it would appear to be of physiological importance. At parturition, estrogen levels are high, thereby aiding in the binding and action of relaxin. Relaxin has also been shown to increase cyclic nucleotide levels in uterine tissue and may in part express its activity on smooth muscle via a cyclic AMP mechanism (8). A specific radioimmunoassay for relaxin has been developed recently (9); this technique in combination with more detailed biochemical studies may aid in elucidating the mechanism of action of this ovarian polypeptide hormone.

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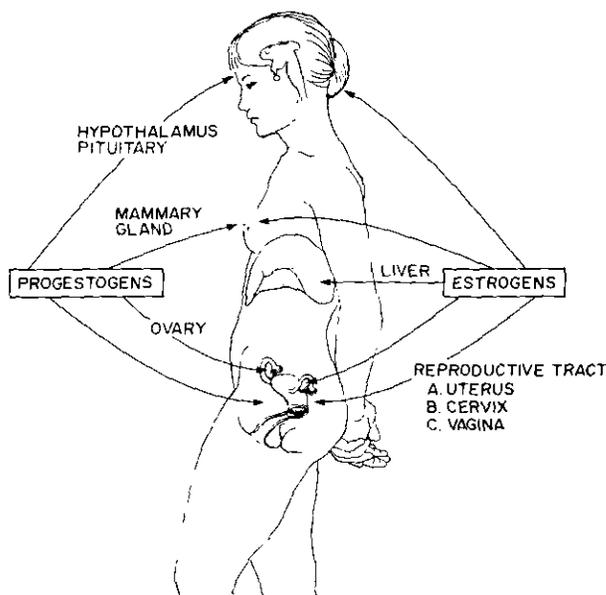


FIGURE 1. Primary sites of action of estrogens and progestogens in the female.

Steroid Hormones

Ovarian steroidogenesis has been studied for many years (10). The role of these steroid hormones and their influence on gonadotropin secretion and ovulation has been reviewed by Ross and Vande Wiele (11). Figure 1 is a generalized view of target sites in the female for the two main types of ovarian steroids. It should be pointed out that these steroids also have effects on secondary and accessory sex structures as well as general body metabolism. An example is premenstrual edema which has been associated with the midcycle and luteal phase increases in estrogen secretion: A cause/effect relationship has been shown to involve an increased retention of sodium, chloride, and water correlated to estrogen levels (12). An exact mechanistic description of this action is complicated by the presence and interrelationships between estrogens and adrenocortical hormones. Estrogens have effects on bone and skeletal tissue. Growth of the long bones is retarded by estrogens acting on the ossification process of the epiphyseal plate. Postmenopausal osteoporosis is believed to be related to the low estrogen levels which can no longer inhibit the bone resorption activity of parathyroid hormone (13).

Neuroendocrine

Neural responses to estrogens and progestogens are customarily involved with the stimulation and/or

inhibition of gonadotropin secretion occurring in hypothalamic and pituitary tissue. Numerous studies have addressed the question of steroid negative feedback at neural centers (14, 15). This topic will be briefly reviewed here, since more detailed descriptions have been published elsewhere (16). Estrogen steroid hormones are believed to be localized because of the high concentration of target cell/neurons in various neural areas such as the pre-optic anterior hypothalamus and the median eminence-basal hypothalamus (17). Specific estrogen receptors have been demonstrated in these hypothalamic areas (18). Functionally, neurons in these areas are influenced by steroids to increase the secretion of releasing hormones, in particular luteinizing hormone releasing hormone (LHRH) (19). This action then results in the stimulation of the anterior pituitary to release gonadotropins (e.g., LH). The exact intracellular mechanism for these actions in the hypothalamus and pituitary is presently unclear. Recent studies using *in vitro* preparations of pituitary cells are attempting to elucidate this mechanism on gonadotropin secretion (20).

Progestogens have been shown to bind to specific intracellular receptors with significant localization in the anterior pituitary and pre-optic anterior hypothalamus (15, 16). Karavolas and associates have suggested that the active form of the progestogens is not progesterone but a 5α - or 5β -dihydroprogesterone (DHP) metabolite (21). This is similar to the actions of androgen steroids and the determination of dihydrotestosterone (DHT) as an active form of this class of steroids in peripheral androgen-responsive organs (22). Although progestogens have not been linked to the negative feedback cycle of gonadotropin secretion, their action in these neurosites has been associated with sexual behavior and the facilitation of ovulation (15).

Mammary Gland and Liver

The mammary gland has received much attention as a steroid target tissue with the recent increased incidence and interest in breast cancer. From our present knowledge, the mammary gland exemplifies the complexity of multihormonal action involved in expression of a physiological function. Ovarian steroid hormones are important compounds in certain aspects of this organ's responses. In noncancerous tissue, estrogen has a specific primary effect in combination with other hormones of stimulating epithelial cells and ductal growth of mammary tissue (23). Progesterone, on the other hand, in the presence of prolactin stimulates the morphogenesis of mammary gland alveoli (24).

There is no apparent action of estrogen in the lobular alveolar structures. This is a clear example of the two ovarian steroids acting independently on specific tissue cell types within a target organ. Normal growth of the mammary gland requires the presence of both estrogens and progestogens as well as additional steroid and polypeptide hormones (25). The phenomenon of lactation involves the action of the steroid hormones in properly developing and preparing the mammary gland for the action of prolactin. Pituitary prolactin is believed to be the hormonal agent responsible for lactation (26).

In recent years, the liver has been studied with regard to endocrine controls. There has been little evidence suggesting an action of progesterone on liver function. The majority of the data and studies have involved estrogen action on liver tissue. Estradiol has been shown to increase specific protein syntheses in this organ independent of a stimulation of DNA synthesis. A variety of liver enzymes such as histidase (27) and serum cholinesterase (28) have been shown to be preferentially synthesized under estrogen administration. The stimulatory action of estrogen on liver protein synthesis is best illustrated by its effect of increasing plasma β -globulins. This class of proteins contains the steroid hormone binding proteins such as corticosteroid binding globulin (CBG) and testosterone-estradiol binding globulin (TeBG) (29). This action in the liver is particularly important throughout pregnancy, during which estradiol functions to increase plasma binding protein concentrations to a sufficient level to maintain a proper free steroid hormone concentration in the blood at a time of high steroid hormone secretion. Estrogen not only stimulates protein synthesis, but can also have an action of inhibiting the synthesis of a particular protein. In this case, estrogen treatment has been shown to decrease the level of α 2u-globulin synthesized in the liver. Detailed studies of this effect have indicated the action involves an actual decrease in the level of mRNA for the protein (30).

In recent years an association between oral contraceptives and liver disease has been indicated. Evidence suggests that the estrogen component of oral contraceptives is possibly related to these abnormalities in hepatic excretory function (31). It is not certain how the estrogens may work to influence liver function, but current studies have indicated an estrogen receptor mechanism present in liver cells similar to those described in other target tissues, such as the uterus or pituitary (32). A more detailed discussion of various controls and programming events in the liver can be found in an additional section of this volume (33).

Ovary

It was mentioned earlier that estrogens have a positive effect on gonadotropin secretion from the pituitary gland (14). There are a number of studies that suggest a stimulating action of the ovarian hormones on various tissue elements of the ovary (34). It has been shown in hypophysectomized animals that estrogens in high doses are capable of stimulating the development of ovarian follicles from small to medium size (35). In particular, the granulosa cells which are found lining the follicle are stimulated by estrogen. Another action of estrogen in the ovary is the enhancement of the effects of gonadotropins (36). The exact mechanism of this enhancement is not clear, but it does not involve an estrogen stimulation or induction of gonadotropin receptors. Rather, the effect is produced by estrogen influences on FSH to stimulate its own receptors as well as those for LH. On the other hand, a direct role for the action of progesterone on the ovary has not been clearly demonstrated to date, but the effect may be indirect via an action in the anterior pituitary on gonadotropin secretion.

Reproductive Tract

One of the main sites of action for ovarian steroid hormones is the reproductive tract of the female. The vagina, cervix, and uterus respond to ovarian steroids in their own individual manner to produce a collective physiological response which is essential for a successful reproductive cycle. We will briefly describe general actions of the two classes of ovarian steroids on these individual organs before discussing the action of estrogens in the uterus in more detail.

In the vagina, estrogen steroids act to increase cell proliferation and stratification of the epithelium which subsequently undergoes cornification. The cornification process results in the appearance of cornified cells in the vaginal smear which can be used diagnostically as an indication of estrogen stimulation. A second primary estrogenic response in vaginal tissue involves the lowering of the pH of vaginal secretions. This acidification occurs by way of an estrogen stimulation of glycogen formation in vaginal epithelial cells. Glycolysis then ensues and the glycogen is converted to lactic acid. The increase in lactic acid in these cells and subsequently in the secretions produces the lower pH. It is believed that these basic actions of estrogen on vaginal tissue provide a defense against vaginal infections. Progesterone action in the vagina is quite different than that of estrogen. Progesterone exposure stimulates mucus secretion. A microscopic

observation of the vaginal secretion will now show few cornified cells as with estrogen, but rather, an increased number of leukocytes present in the secretion.

In contrast, the actions of the two types of steroids are quite different in cervical tissue. Estrogen stimulates mucus secretion which is of low viscosity and exhibits an alkaline pH. Presumably, this aids in sperm transport and survival resulting in increased reproductive success. Progesterone produces a reduction in the amount of mucus secreted, in addition to a thickening of the secretion. This, in fact, was thought of as a possible contraceptive mechanism whereby progesterone agents could be given to increase the viscosity of the cervical mucus resulting in a retardation of sperm motility and penetrability in the female reproductive tract.

One of the most dramatic effects of steroid hormones on mammalian reproductive tract tissue occurs in the endometrial lining of the uterus. In the case of estrogen, laboratory animal studies have indicated that stimulation appears to occur in two phases: an early phase from 0 to 6 hr and a second growth phase from 6 to 24 hr (37). This later mitogenic response involves both hypertrophy and hyperplasia responses within the tissue (38). The size and weight of the uterus increases some 3- to 4-fold over the nonstimulated state. There is an increase in cell number with the luminal epithelial cells changing from a cuboidal to a columnar type of cell. General cellular metabolism is increased with a variety of enzymes such as glucose 6-phosphate dehydrogenase (39) being specifically induced. Water imbibition occurs to a marked extent, and there is pronounced hyperemia in the tissue. Finally, the tissue shows significant increases in RNA and DNA synthesis with mitosis occurring by 16-24 hr after estrogen stimulation. In general, progesterone acts in the rodent and primate uterus to change the estrogen induced proliferative endometrium into a secretory endometrium (11); this also results in inhibition of any further luminal epithelial cell proliferation (40). Such a physiological change in the tissue aids the endometrial lining in nidation of the fertilized ovum. If pregnancy does not ensue then menses results. The exact intracellular mechanism for this progesterone action in the uterine endometrium is unclear, but estrogen stimulation is a prerequisite to induce progesterone receptor. The progesterone acts to limit the estrogen receptor replenishment (41), thereby minimizing the estrogen effect on the tissue. In certain species, such as the dog, cat, and rabbit, uterine growth is stimulated by progesterone rather than estrogen (42). The experimental evidence involved with estrogen

GENERALIZED MODEL OF ESTROGEN ACTION IN TARGET CELLS

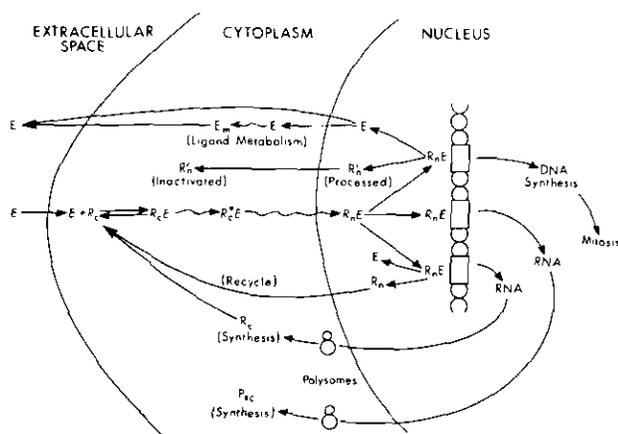


FIGURE 2. A generalized model of estrogen hormone action in uterine target cells.

stimulation of uterine growth has come primarily from studies using the rat uterus. There have been several reviews which have concentrated on this species (37, 43).

Mechanism of Intracellular Action of Estrogen

A generalized model for the intracellular action of estrogen hormone is diagrammatically illustrated in Figure 2. The hormone enters the cell through passive diffusion and interacts with a cytoplasmic receptor exhibiting a high binding affinity for the hormone (44). The hormone-receptor complex then undergoes a change in physical state to an "activated" form (45). In this "activated" state, the hormone-receptor complex can then translocate to the nucleus of the target cell. Once inside the nucleus, the complex is believed to interact with an acceptor site(s) at specific areas of the genome. A precise description of the nuclear interactions of the hormone complex as well as the molecular biology of gene expression in uterine tissue is presently incomplete. The reader is directed to studies using the chick oviduct as a model system for these research areas (46, 47).

Following the interaction in the genome, specific messenger RNA is transcribed with subsequent translation to specific proteins. As mentioned earlier, glucose 6-phosphate dehydrogenase is an example of such an action (39) in addition to a protein termed IP or "induced protein" (48). After such initial RNA and protein synthetic events occur, the general rate of cellular activity increases as out-

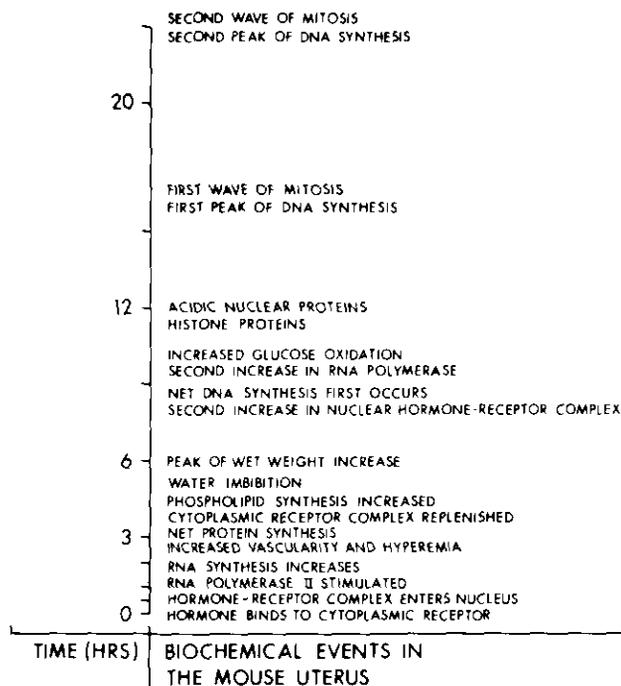


FIGURE 3. A temporal pattern of biochemical events which occur in the mouse uterus after estrogen stimulation.

lined in Figure 3. Hormone-receptor complex is continuously lost from the nucleus after the initial interaction at approximately 1 hr. The receptor level in the cytoplasm is then replenished through recycling and synthesis (41). Induction of progesterone receptor by estrogen is also occurring in the cell at this time and has been clearly documented (49). These events, in addition to an increase in nuclear proteins and DNA synthesis finally results in mitosis and cell division.

As mentioned earlier, the rodent uterus, in particular, the rat uterus, has been studied extensively as a model system for certain aspects of estrogen action. A question which commonly arises in endocrine and/or toxicological studies involves the best choice of species for conducting certain experiments. Since the mouse has been utilized in various toxicological (50) and carcinogenic (51) studies and since the responses in the rat uterus have been previously summarized (43), it seemed worthwhile, in this chapter, to summarize the normal biochemical responses of the mouse uterus. Hopefully, this may be of some aid and reference to other investigators using this species. In Figure 3 is a temporal listing of various biochemical events which have been described in the mouse uterus after estrogen stimulation. These data come from a number of studies both in this laboratory (52-55) as

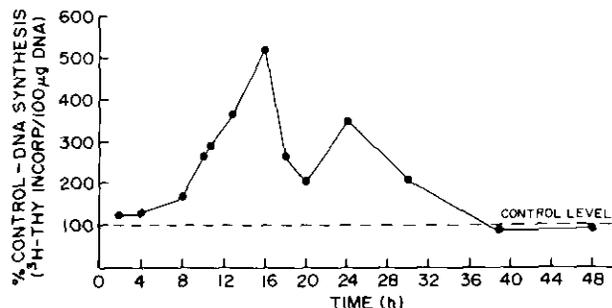


FIGURE 4. Temporal pattern of DNA synthesis measured in the castrate mouse uterus after a single IP injection of 20 µg/kg estradiol. DNA synthesis was quantified by the procedure outlined by Harris and Gorski (57).

well as those of others (38, 40, 42). Certain responses, such as the early events involving the receptor, water imbibition, wet weight increase, and increased vascularity and hyperemia appear to be similar in most species. However, there are also some species differences in the cellular response to estrogens. For example, in the CD-1 mouse, there is a second nuclear increase in hormone-receptor complex (52), which appears to be related to the late growth response (53), but clear evidence of such an increase in the rat is not presently available. One of the most significant endpoints of estrogen action in the uterus involves stimulation of DNA synthesis. Figure 4 shows the temporal pattern of DNA synthesis in the castrate mouse uterus following a single injection 20 µg/kg estradiol. A significant net increase in synthesis occurs around 6-8 hr, while in the rat this increase occurs around 12 hr (43). There are two peaks of DNA synthetic activity occurring; the first is at 16 hr and quantitatively is the largest; a second and smaller response occurs at 24 hr. These two peaks of DNA synthesis are also accompanied by two waves of mitosis (56) which occur at approximately the same time. Studies using the rat uterus have not indicated similar findings.

The biphasic nature of the responses shown here for the mouse uterus may be related in part to the differential responses seen in the stromal and epithelial cell compartments (38, 40). Those studies illustrated in adult castrate animals that estrogen stimulates cell division primarily in epithelial cells even though steroid hormone was localized in the nuclei of both cell types. When this localization was further investigated as a temporal event, preliminary studies using autoradiographic techniques demonstrated a differential nuclear uptake of hormone in the mouse uterine tissue (58). The glandular epithelium and stromal cells showed nuclear localization early after hormone administration (1

hr) while luminal epithelial cells did not show significant nuclear accumulation of hormone until 7-8 hr. One explanation for such findings may be the requirement for hormone interaction in one cell compartment (stroma/glandular epithelium) which then influences hormone uptake and/or response in another cell compartment (luminal epithelium). Whether this mechanism involves the synthesis of some factor awaits further experimentation; although, the existence of tissue factors have been suggested from studies investigating the differentiation and development of reproductive tract tissue (59). In those studies using tissue recombinants it was shown that the stromal cell compartment had influence on its overlying epithelium. Therefore, it seems reasonable to expect that future studies both toxicological and/or endocrine in nature, should consider that target organs may exhibit a different responsiveness and/or effect in various tissue compartments.

Summary

This review has intended to describe the basic sites of action and the diversity of responses induced by ovarian hormones. In addition, the detail and complexity of target organ responses, as well as the differences in animal species responses to the hormone have been illustrated. Hopefully, an appreciation of these points may be useful in understanding various toxicological problems as well as the actions of environmental compounds which may possess similar hormonal activity.

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