Artykuł przeglądowy *Review*

Role of hormones and growth factors in initiating and maintaining the lactation of seasonal animals

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Summary

In seasonal animals, the process of triggering and maintaining lactation requires numerous hormones. The interaction of growth factors and other hormones is necessary in processes such as mammogenesis, lactogenesis and galactopoesis. Due to the proper synchronization of pregnancy and changes in the area of the mammary gland, the gland is ready for the production of milk at the moment the offspring is born. Mammogenesis is a phenomenon that requires the participation of a number of hormones, including prolactin (PRL), growth hormone (GH), estrogens, progesterone, oxytocin, placental lactogen (PL) and insulin-like growth factor (somatomedine, e.g., IGF1). The co-participation of IGF and GH is necessary in coordinating the differentiation and proliferation of epithelial cells. The manner in which the growth factors stimulate or inhibit the growth of cells or their influence on the cell cycle is not fully understood. The role of IGF in particular stages of functioning of the mammary gland (mammogenesis, lactogenesis, galactopoesis, desiccation), particularly in the case of ruminants, is highly complicated. Recently, attention has been given to the metabolic hormones, particularly the role of leptin in mammogenesis, lactogenesis and galactopoesis. The discovery of the leptin mRNA in mammary gland cells of many species, including humans, suggests that the mammary gland in itself may be the structure producing leptin. Leptin is the paracrine factor that influences the growth, development and functioning of the mammary gland by interacting with leptin receptors in areas of epithelial tissue. Due to the recently increased interest in sheep's milk products, an understanding of the endocrine mechanisms facilitating the maintenance of lactation during autumn and winter may contribute to the improved profitability and usefulness of sheep's milk.

Keywords: mammary gland, lactation, prolactin, metabolic hormones

The reproductive season of many species is closely related to the time of the year and specific environmental conditions. To ensure the greatest possible chances of offspring survival, adaptation mechanisms that are designed to synchronize the endogenous reproductive cycle of the species to changing environmental factors have evolved. Changes in day length constitute a key factor regulating reproductive cycle progression in sheep (13). The reaction of an organism to the lengthening or shortening of the day light phase is the result of the changes in the concentration of melatonin produced in the pineal gland under dark conditions. Information about the length of the day is transferred to the body through the retinal-hypothalamic pathway. The signal, which results from the interaction of the light stimulus with the retina of the eye, reaches the even-numbered clusters of neurons, the so-called hypothalamic suprachiasmatic nuclei (SCN), the paraventricular nucleus (PVN), a roll of the upper cervical spinal cord, and finally the pineal gland, where it is converted into a hormonal signal in the form of melatonin (12).

In the case of sheep, which are short day animals, melatonin induces pro-gonadotropic action and acts as a stimulant for the secretion of gonadotropic hormones from the pituitary gland. This effect occurs within the central nervous system and manifests itself in the intensification of GnRH (gonadotropin releasing hormone) secretion and the increased secretion of LH (luteinizing hormone) and FSH (folliculotropin stimulating hormone). These hormones signal the start of the reproductive cycle and trigger a number of hormonal interactions (26). In the case of a pregnant female, these processes prepare her body for rearing offspring. The onset of the development of the mammary gland (mammogenesis) and the processes of the initiation (lactogenesis) and maintenance of lactation (galactopoesis) are fully under the control of the endocrine system. The endocrine system plays a key role in the synchronization of the development of the mammary gland and its subsequent operation, depending on the degree of puberty of young animals and the offspring's need for food (21).

The roles of GH, IGF and insulin in mammogenesis

Mammogenesis is a phenomenon that requires the participation of a number of hormones including prolactin (PRL), growth hormone (GH), estrogens, progesterone, glycocorticoid, oxytocin, placental lactogen and an insulin-like growth factor (somatomedine, e.g., IGF1). Somatomedins belong to a group of metabolic hormones and coordinate the body's response to metabolic changes and stress factors (5).

The development of the mammary gland has several phases, each of which occurs under the control of specific hormones. The first stage, conduction morphogenesis, requires the participation of estrogens, GH and several growth factors such as TGFβ, IGF1 and amphiregulin, which are present in the mammary gland, to stimulate its development (32). The activity of GH is the most visible during the conduction morphogenesis. The binding of GH to framework receptors induces the secretion of IGF1 from the follicular epithelium in a paracrine way (5). The proliferation phase of follicular morphogenesis occurs under the influence of progesterone and prolactin. The identification of growth factors such as EGF (epidermal growth factor) and IGFs in milk suggests that these growth factors are synthesized and transported through the epithelium, which is responsible for the production of milk. The results of previous studies indicate the significant participation of these growth factors in regulating the growth and morphogenesis of the mammary gland.

The co-participation of IGF and GH is necessary in coordinating the differentiation and proliferation of epithelial cells. It has been proven that IGF acts as a mediator for GH during mammogenesis in rodents (31). The main source of IGF1 is the liver, which produces this factor that affects almost all tissues in an organism, including the mammary gland. IGF is most likely the mitogenic factor that is involved in the processes of proliferation and apoptosis. IGFs interact with the cells through the receptors called IGF receptors type 1 (IGFR-1) (19).

In vitro studies have shown that insulin has a significant effect on the maintenance of normal histological structure and the functioning of the mammary gland. Insulin affects the growth and metabolic processes of living organisms. This hormone regulates glucose consumption (for energetic purposes) and the synthesis of lactose, milk fats and casein. Insulin increases the fat content in milk by stimulating long chain fatty acid synthesis. In ruminants, insulin enhances milk yield. The presence of insulin is essential for the interaction of GH with the cells of the mammary gland because it activates the IGF1 receptors. Further studies have shown that IGF1 stimulates both the growth of mammary epithelial cells in vitro and the synthesis and secretion of IGF-binding proteins (IGFBPs) in cells that are responsible for the production of milk in sheep and mice. In the mammary tissues of sheep, mRNA for IGF1, IGF2 and IGFBPs was detected, which constitutes at least partial evidence for the origin of these compounds from the previously mentioned gland. The manner in which the growth factors stimulate or inhibit the growth of cells or their influence on the cell cycle is not fully understood.

The role of estrogens and placental lactogen in lactation

The development of the mammary gland is a long-term process that requires estrogens. The bud of the mammary gland and teats and the glandular sinus are already present in newborn animals. When animals reach sexual maturity, mammogenesis of the duct system (ductal mammogenesis) occurs in the presence of estrogens and progesterone. It has been demonstrated that mammogenesis was inhibited in cows that had an ovariectomy procedure. However, the administration of exogenous estrogens caused that process to resume. Estrogens also have a positive effect on ductal and lobuloalveolar mammogenesis. The presence of both progesterone and estrogens are essential for the proper development of the mammary gland. It has been shown that estrogen receptors are present in the mammary gland; therefore, estrogens have a direct impact on its tissues. An indirect effect of estrogens is the stimulation of PRL release from the pituitary and an increase in the number of PRL receptors present in the mammary gland. Moreover, estrogens cause the increased secretion of growth factors (IGF-1, TGF-α) and the increased sensitivity of glandular cells. However, the exact role of estrogens in mammary gland development is not fully understood (7).

The presence of estrogens is necessary for lactogenesis because they sensitize mammary glands to lactogenic hormones. However, if the estrogen concentration is high, milk production decreases. The key factor for lactation initiation is an appropriate estrogen to progesterone ratio. During pregnancy, the level of progesterone remains quite high, which inhibits the stimulatory effect of estrogens on PRL function. A decrease in the progesterone concentration around the perinatal period results in the greater secretion of PRL and GH. Moreover, the mammary gland, sensitized to lactogenic hormones by estrogens, increases milk synthesis.

The mammogenic activity of placental lactogen has been demonstrated by in vitro studies on sheep mammary gland tissue. In vitro it has been observed that the gland tissue responds weakly to ovarian steroids (progesterone, 17β-estradiol). However, incubation with these steroids for 2 days sensitized the epithelial cells. As a result, the addition of PL, cortisol and insulin or PRL, cortisol and insulin caused the development of milk duct cells in glandular tissue. In vitro experiments have shown that PL and PRL complete the other's functions. However, the presence of PRL and GH is required for optimal mammary gland development. The somatotropic activity of PL seems to be sufficient to stimulate gland development. In pregnant sheep, the multiplication of epithelial cells occurs normally, even in the absence of PRL. An indirect effect of the placenta (PL secretion) on the mammogenesis process has been demonstrated in sheep. In the first trimester of pregnancy, the sheep underwent hypophisectomy, i.e., the hypothalamus-pituitary junction was disconnected. As a result, the development of mammary glands was normal in only 40% of the animals. The same results were obtained for pregnant goats. The hypophisectomised animals showed adrenal gland atrophy and lacked GH and ACTH. Mammary gland development was significantly different between these animals and those with a low PRL concentration. The activity of placental lactogen may explain why sheep pregnant with twins produce 30% more milk than do sheep with a single pregnancy. Sheep and goats show a positive correlation between PL secretion, mammary gland weight, milk yield and the number of offspring (22).

The role of leptin in the lactation process

Recently, researchers' attention has turned to metabolic hormones, particularly the role of leptin in the processes of mammogenesis, lactogenesis and galactopoesis. Leptin is a hormone produced by fat cells (adipocytes), primarily in the subcutaneous white adipose tissue. The hormone plays a role in the energy management processes of the body and regulates food intake (6). This hormone is involved in various processes, including control of the reproductive system, the endocrine system, metabolism of tissues, blood pressure, hematopoesis, and angiogenesis. It also impacts the changes in the PRL and GH concentrations and controls the steroidogenesis process. The concentration of leptin is higher in the blood of females than it is in males, which is related to the estrogen level. The amount of leptin in the blood in the follicular phase is proportional to the amount of estradiol. The study concerning leptin focuses mainly on obesity. Obesity is a risk factor for the occurrence of mammary cancer in women and mice, particularly after menopause. Along with an increase in body weight and adipose tissue, there is a significant increase in serum leptin. As previously mentioned, cytokines act through long forms of leptin receptors known as OB-Rb. This study has shown that leptin, acting through receptor OB-Rb has a significant impact on the proliferation of mammary epithelial cells (17).

The discovery of leptin in milk raises the question of whether mammary epithelial cells are merely involved in the transfer of the PRL, GH and cytokines from the blood or whether they are the source of these substances (9). The answer was obtained in an experiment using lactating rats that resulted in the detection of leptin in milk after the previous intraperitoneal injection of that substance, which confirmed the assumption of the role of the cells in transport (8).

The discovery of leptin mRNA in mammary gland cells of many species, including humans, suggests that the mammary gland itself may be the structure producing leptin. Leptin receptor mRNA has been detected in mammary epithelial cells in sheep (20). That study showed that the expression of leptin mRNA occurs during lactation in the tissue of mammary gland of sheep (2). Leptin gene expression is strongly correlated with the stages of pregnancy and lactation. It has been proven that the level of leptin mRNA is significantly lower during the feeding of offspring than it is during pregnancy. During the full stage of cell differentiation, the amount of body fat is reduced (5). The changes in leptin secretion during pregnancy and lactation are the result of different numbers of adipocytes in the mammary gland. The expression of genes reaches its maximum intensity at the beginning of pregnancy and is reduced at the end of pregnancy. Leptin is a paracrine factor that influences the growth, development and functioning of the mammary gland by interacting with the leptin receptors in areas of epithelial tissue (5).

Prolactin and the mammary gland

The process of mammary gland differentiation leads to the initiation of lactation (16). Prolactin is the main hormone responsible for changes in the mammary gland areas during mammogenesis and full lactation. High concentrations of PRL stimulate secretion in the cells responsible for the production of milk, while oxytocin aids in the extraction of milk from the milk ducts. Prolactin is a peptide hormone that is composed of amino acids that form a single chain. Secretion occurs in acidophilic lactotropic cells of the anterior pituitary. The incidence of prolactin and the receptors of that hormone have also been detected in part of the neurohypophysis and in structures of the limbic system such as the amygdale, hypothalamus, and hippocampus (14). In addition, prolactin is produced in cells of the immune system, placenta and uterus muscle membrane (3). PRL synthesis may also occur in the cells of certain cancers (7). The number of lactotropic cells is always higher in females than in males; during pregnancy, the number and size of cells increase. The concentration of PRL in the body of females exceeds the content of this hormone in males, which is related to the stimulating activity of estrogen. The control of PRL secretion is mediated by substances that originate from the central nervous system. Angiotensin II shows a stimulatory effect on PRL-secreting lactotrophs; angiotensin is a hormone-releasing thyroid hormone and vasoactive intestinal peptide. Oxytocin, bradykinin, bombesin, and neurotensin may also have a stimulating effect. In the case of substances that suppress PRL secretion (e.g., PIF – prolactin inhibiting factor), the neurotransmitter of a group of catecholamines synthesized in dopaminergic neurons deserves special attention. The synthesis and secretion of dopamine takes place in the area of the hypothalamus. Dopamine produced in neurons (TIDA – tuberoinfundibular dopaminergic neurons) is the main controller of PRL secretion. Dopamine D2 receptors reside on the cell membrane of lactotrophs, to which the dopamine molecule binds (10). The hypothalamus produces substances that suppress PRL secretion independently of dopamine, including regulatory protein (GAP – GTPase activation protein), DKP (diketopiperazyno-histidine-proline) and GABA (γ-aminobutyric acid).

Prolactin interacts with the target cells through prolactin receptors. The presence of these receptors was found in the ovaries of sheep, swine and mice (11, 29). The distribution of PRL receptors depends on the number of amino acids in the molecule. Prolactin is not a homogeneous hormone but is divided into isoforms. Each isoform impacts the target cells that connect it to the appropriate receptor. Thus, the form "little" connects itself to the receptor PRLr – RS, the form "big" connects itself to the receptor PRLr – RI, and the form "big – big" connects itself to PRLr – RL (18). The signal generated due to the merging of prolactin molecules with the receptors is not equally strong for all isoforms. The most intense effect is induced by molecules that are connected to long-form receptors (30).

In organisms with marked seasonality, the important factors that regulate the secretion of prolactin are thyroid hormones, estrogen and GH, insulin, and glucocorticoids. These hormones affect the prolactin gene transcription process. This process is inhibited by T3 (triiodothyronine). In the case of estrogens, their activity is manifested by an increase in the number of lactotropic cells of the pituitary and the increased sensitivity of lactotrophs to TRH (thyroid releasing hormone) and dopamine.

Similar to prolactin, GH is formed in the adenohypophysis. It plays a role in the synthesis of proteins and fatty acids and reduces the concentration of glucose in the blood. It is partially responsible for the synthesis and secretion of PRL (25). A stimulus in the form of suction can increase GH secretion. Increased levels of this hormone are observed in early lactation. In sheep, PRL secretion and GH are dependent on changes in the length of daylight and are related to the different levels of melatonin synthesized in the pineal gland (13). Periodic changes in the concentration of melatonin result in the rhythmical inhibition of PRL secretion (35). During lactation, the levels of PRL and GH in the blood significantly increase due to the suckling stimulus (25). Growth hormone secretion influenced by the suction effect is controlled by GHRH (growth hormone releasing hormone) and endogenous opioids (23). Recently, attention has been given to salsolinol, a compound derived from dopamine. An increased concentration of this substance was found in individuals suffering from dysfunction of the dopaminergic system (1). Salsolinol intensifies PRL release in the case of rodents and ruminants in a variety of physiological conditions (15, 34). The presence of salsolinol was confirmed in the hypothalamus (MBH – mediobasal hypothalamus) in lactating sheep, and its concentration increased in response to suction (24). The injection of salsolinol to the third ventricle of the brain causes an increase in the concentration of PRL in animals in the lactating phase. 1MeDIQ is an antagonist of salsolinol (28). This compound inhibits the release of prolactin and eliminates the suction effect on PRL secretion in rats (4). This phenomenon has also been observed in sheep, in which 1MeDIQ directly affects the central nervous system (24). The antagonist of salsolinol 1MeDIQ influences the reduction of the GH concentration in blood, indicating its direct effect on the CNS (central nervous system). This compound inhibits the increase of the NA (noradrenaline) concentration. The previously mentioned catecholamine is a mediator between salsolinol and GH (28). Interestingly, 1-MeDIQ does not affect the change in GH content that is induced by suction. The standard content of PRL in sheep outside the period of suckling lambs and during nursing was decreased after the application of 1-MeDIQ (24). These observations indicate that salsolinol has no direct effect on GH secretion during suckling. In rats treated with salsolinol and 1-MeDIQ, no significant changes were observed in the concentration of pituitary hormones, except for PRL (33).

Prolactin profile changes have a significant impact on milk production parameters. In cows, an increased level of prolactin results in an increased content of α-albumin in milk. In turn, the milk of cows in which the decreased secretion of PRL has been proven contains significantly less fat, protein and lactose. The results of a chemical composition study of sheep's milk showed that the content of dry matter, protein, fat and lactose was slightly higher in the breast milk of animals born in June compared with those born in January (27). In the case of mares, the milk has better parameters with a strong increase in PRL secretion. The process of triggering and maintaining lactation requires the presence of numerous hormones. Thus, in sheep, which are seasonal animals, it is difficult to provoke induced lactation and keep milk secretion during the shortening of the day. Due to the increased interest in sheep's milk products, an understanding of the endocrine mechanisms facilitating the maintenance of lactation during autumn and winter may contribute to the improved profitability and usefulness of sheep's milk.

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