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Effect of High Energy Intake and Treatment with Galanin on Gonadotropin Levels in Female Goats

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ARTICLE INFO	A B S T R A C T
Article Type: Original Article	 Background: Galanin is an orexigenic agent and has been demonstrated to be a putative regulator of gonadotropin secretion. It is well known that this orexigenic peptide probably plays a vital role in the regulation of reproduction; however, the underlying mechanisms have not been fully elucidated. Objectives: The goal of this study was to determine the role of galanin in the regulation of gonadotropins in goats, which were subjected to either high or low energy diets. Materials and Methods: Adult female Saanen goats were randomly divided into 2 groups. Animals in the two groups were fed either a 100% or a 150% energy content diet, respectively, for a month. On the first day of the experiment, all animals received an intravenous injection of 1 µg galanin/kg body weight. On the second day, each animal was administered 2 µg galanin/kg body weight. Blood samples were withdrawn by jugular venipuncture at 30-minute intervals, 3.5 h before and 3.5 h after galanin injection. In order to determine the concentration of LH and FSH, plasma was isolated and subjected to a high-energy feed significantly reduced circulating LH and FSH levels. It contrast, this dose had no effect on goats subjected to the lower energy diet. The injection of galanin at a dose of 2 µg/kg body weight had no influence on mean plasma concentrations of gonadotropins in goats fed either of the two diets. Conclusions: The results indicate that galanin is not a principal hypophyseal regulator in the secretion of gonadotropins in Saanan goats. It may be inferred that in the same animal model galanin has a gonadotropin-reducing effect when its in vivo concentration increases.
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▶ Implication for health policy/practice/research/medical education:

High energy intake and peripheral galanin injection could decrease gonadotropins secretion. Thus, it can be concluded that galanin is a metabolic signal for GnRH-LH/FSH axis in high-energy feed goats.

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1. Background

Galanin is a 29-amino acid peptide that was originally isolated from porcine intestine (1). It is found in the central and peripheral nervous systems as well as in the anterior pituitary (AP) gland . Galanin has several physiological functions, including food intake, body weight maintenance, hormone release, and reproduc-

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tion (2). Three galanin receptors have been identified, namely, galanin receptor-1 (GAL-R1), galanin receptor-2 (GAL-R2), and galanin receptor-3 (GAL-R3) (3).

There have been several previous investigations on the effects of galanin on the regulation of gonadotropin secretion, particularly in rats. The results of these studies have revealed that galanin enhances the binding of gonadotropin-releasing hormone (GnRH) to pituitary membranes (4). In addition, the intracerebroventricular administration of galanin stimulates the release of LH (5). In contrast, the administration of specific galanin antiserum (galantide) suppresses LH release (6). Some studies have shown that galanin can act as an inhibitor of gonadotropin release. For example, galanin has been shown to inhibit GnRH-stimulated LH secretion from rat pituitary fragments (7).

In vitro studies have revealed that galanin can stimulate the basal release of LH from porcine AP cells (8); however, another study has shown that isolated anterior pituitary cells from male rats are not influenced by exposure to galanin (9). Furthermore, galanin was shown to have no effect on the secretion of LH in humans of either sex (10, 11). It is already well known that a relationship exists between the energy status and the reproductive function of an organism. However, the underlying mechanisms of this relationship have not yet been fully investigated. Hormonal signaling chemicals such as leptin, ghrelin, orexins, and neuropeptide Y (NPY) are involved in the control of reproduction and energy homeostasis (12).

Galanin is known to play a role in the control of energy homeostasis. When galanin is injected into the hypothalamus, it has been shown to stimulate food intake and reduce energy expenditure. In addition, nutrient ingestion or the presence of other circulating hormones can influence the production of this peptide in the hypothalamus. For example, insulin and leptin inhibit galanin gene expression and peptide immunoreactivity (IR) in the hypothalamus (13). With respect to ruminants, the role of galanin in the regulation of the GnRH/LH system is not well understood, and the affect of galanin treatment on the feeding status of such mammals has, as yet, not been studied. The role of galanin as a regulator of pituitary reproductive hormones in ewes is supported by the presence of galanin-immunoreactive neurones in the medial preoptic area and the infundibular nucleus (14). In addition, GAL-R1 has been found to be expressed in GnRH neurons (15).

2. Objectives

The purpose of the current study was to determine whether the peripheral injection of galanin can affect the mean plasma concentrations of LH and FSH in female Saanen goats that were fed diets of different energy contents.

3. Materials and Methods

3.1. Animals

The adult female Saanen goats (60 kg) used in this study were housed individually in the Yazd Research Center for Agriculture and Natural Resources. The study was performed in the fall season. Goats were housed under an 11-h dark/13-h light photoperiod. Only female goats that had completed an estrous cycle were included in the study.

3.2. Experimental Design

Female Saanen goats were randomly selected from a flock and divided into two groups. Animals in the first and second groups were fed a 100% (NE) or 150% energy (HE) content diet, respectively. To determine the roles of galanin (Sigma USA) on gonadotropin secretions in goats fed diets of different energy contents, 1 month after initiation of the dietary regimen, the goats received intravenous injections of either 1 or 2 µg galanin/kg body weight (BW) into their jugular vein. Intravenous injections of galanin were used in order to restrict target sites within the hypothalamic-pituitary axis to those outside the blood-brain barrier. Blood samples were collected by jugular venipuncture at 30-min intervals (16), 3.5 h before and 3.5 h following galanin injection. The samples were then centrifuged and the plasma was stored at -20°C until assayed for LH and FSH levels. The animals received one of the two galanin treatments on separate days. Blood samples that were collected before the injection of galanin in the two treatments served as the controls for each group.

3.3. Feeding

Gross energy and chemical compositions of feedstuffs, namely, dry matter, crude protein, crude fiber, ether extract, total ash, NDF, ADF, calcium, and phosphorous, were analyzed in the Yazd Research Center for Agriculture and Natural Resources. Animals were maintained on the diets for 30 days. Diets were formulated based on maintenance energy requirements and according to Ensminger tables (17). Diet 1 (2.4 Mcal) consisted of 200 g molasses, 200 g refuse beetroot, 700 g barley, 700 g alfalfa, and 100 g refuse soya (41%). Diet 2 (2.58 Mcal) consisted of 100 g molasses, 100 g fat powder, 200 g refuse soya (41%), 500 g barley, and 900 g alfalfa. The goats had free access to fresh water.

3.4. Hormone Assays

Plasma concentrations of LH and FSH were determined by double-antibody radioimmunoassay (RIA) with standard LH and FSH ovine antigens (Tabeshyarnoor Co., Iran). The intra- and interassay coefficients of variation for LH and FSH were 6%, 4%, 9%, and 6%, respectively.

3.5. Statistical Analyses

ANOVA was used to assess any statistical differences in mean LH and FSH levels in animals in response to galanin treatments (1 or 2 μ g galanin/kg BW) within a predefined diet treatment group (100% or 150% dietary energy content). All statistical analyses were performed using SPSS (version 13). The results are shown as means ± SE and the level of significance was set at p < 0.05.

4. Results

The results of the study are shown in Figures 1 and 2. The ANOVA analysis failed to reveal a statistically significant difference between the mean concentrations of LH and FSH before and after the injection of 1 μ g/kg BW galanin in goats fed the 100% energy diet. However, the mean concentrations of LH and FSH were significantly decreased



Figure 1. LH Concentration in Goats before and after the Administration of 1 or 2 μ g/kg BW Galanin. * p < 0.05

Data is presented as the mean \pm SE.



Figure 2. FSH Concentration in Goats before and after the Administration of 1 or 2 μ g/kg BW Galanin. * p < 0.05

Data is presented as the mean \pm SE.

following the administration of galanin in goats that were fed the 150% energy diet (p < 0.05). The administration of 2 µg/kg BW galanin had no significant effect on the mean concentration of gonadotropins in goats that were fed the two different diets.

5. Discussion

Galanin has been reported to regulate the secretion of gonadotropins in rodents. In vivo and in vitro studies have indicated that galanin exerts its effects on the reproductive axis at two different levels, namely, the hypothalamus and the pituitary (2). Our results indicate that galanin does not play an important role in the regulation of gonadotropin secretion in goats, at least at level of the pituitary gland. To the best of our knowledge, this is the first study in which the effects of galanin on gonadotropin secretion in goats fed diets of two different energy levels have been investigated. Only the administration of 1 µg/kg BW galanin in goats that were fed the higher energy diet appeared to influence the secretion of gonadotropins. More specifically, the lower dose administration of galanin in this scenario appeared to decrease the mean plasma concentration of LH and FSH by 36% and 37%, respectively. These results are similar to previously reported data. Schmidt et al. reported that the injection of NPY in rats that were administered food-restricted diets stimulated the secretion of LH. In contrast, the authors claimed that this was not the case in control rats that were fed ad libitum. The authors suggested that rats that are diet-restricted are more sensitive than control rats to the physiological effect of NPY (18). Sirotkin et al. demonstrated that in fed rats the administration of ghrelin only induced a trend towards decreased testis mass without detectable changes in the final plasma levels of gonadotropins and testosterone. In contrast, in food-restricted animals, in which endogenous ghrelin levels are known to be increased, chronic administration of ghrelin induced significant decreases in plasma LH and dramatically suppressed testosterone levels. Thus, it was postulated that the effect of ghrelin on LH secretion is dependent on the nutritional state (19). The results of the current study suggest that the effect of galanin on gonadotropins secretion in goats depends on the metabolic state and energy balance of the animal. Most probably, the increase of energy in the diet can change the sensitivity of gonadotropins secretion toward galanin. The administration of galanin in goats who were fed an energy-rich diet (150% dietary energy) appeared to decrease the concentrations of circulating LH and FSH. Moreover, galanin neurons have noticeable differences compared with those of other orexigenic peptides such as NPY (20). Unlike NPY expression, which is increased in the restricted energy state (21), galanin expression remains unchanged under the same conditions (22). Highfat diets have been shown to induce galanin gene expression in the hypothalamus (13). In addition, the number of galanin neurons increases in overfed weanling rats (23). In this study, the expression of galanin and its resultant concentration changes in the plasma were not investigated. It is possible that endogenous galanin levels in goats administered high-energy (150% dietary energy) diets increased, and that the summation of exogenous and

endogenous galanin levels resulted in the decrease in gonadotropin secretion. It is possible that galanin plays a dampening role at high-energy status and regulates the pituitary response to the increased GnRH. This result is similar to the previously published findings. Finn et al. demonstrated that although galanin can stimulate LH release in primates, high concentrations of this peptide were required (24).

The binary effect of galanin can depend on the presence of galanin receptor subtypes in the hypothalamus-pituitary axis. GAL-R2 and GAL-R3 have been reported to be expressed in the anterior pituitary and the activation of the GAL-R3 receptor subtype has been shown to cause hyperpolarization and reduced exocytosis (3). Thus, it is possible that in goats exposed to high-energy diets (150%), the expression of GAL-R3 in the anterior pituitary predominates and this may lead to decreased plasma concentrations of LH and FSH. In addition, the inhibitory action of galanin toward gonadotropin secretion could be due to the presence of galanin receptor subtypes in the median eminence of the brain. Galanin decreases the release of GnRH or that of another hypothalamic factor from nerve terminals in the median eminence, and thereby indirectly causes a decrease in the pituitary secretion of LH and FSH. This anatomical area is outside the blood-brain barrier, and therefore it is possible that even large and/ or polar metabolic cues and hormones could potentially interact directly with neurones in this region. There is some experimental evidence to support the notion that metabolic cues can act within or close to this anatomical region, and thus regulate neuronal activity. For example, insulin and cholecystokinin receptors have been detected in the basal region of the hypothalamus (25). In addition, studies in ewes have indicated that GAL-R1 is expressed in GnRH neurons (15) and that galanin-immunoreactive neurones are present in the median eminence (14). Circulating plasma leptin concentrations have been shown to be positively correlated with positive energy balance and leptin increases the secretion of LH (26). The intravenous administration of galanin decreases the plasma concentration of leptin (27). Thus, it is suggested that high concentrations of galanin in the 150% energy diet (but not in the 100% energy diet) decreased plasma leptin, and that galanin thus indirectly decreased LH and FSH via leptin.

In goats that were fed the lower (100%) energy diet, the injection of galanin had no effect on the secretion of ganadotropins. However, the intravenous administration of galanin in female rats has been shown to increase LH secretion (28). This discrepancy can possibly be explained by species-related differences in galanin function (8) and also differences in experimental methodology. It has already been documented that the inherent function of some peptides such as NPY is species-dependent. In rodents and sheep, NPY increases and decreases the secretion of LH, respectively (9,21). Further, whilst galanin has been shown to stimulate the secretion of growth hormone (GH) in rats, it has no effect on the secretion of this hormone in pigs (8). In humans, in spite of the synaptic contacts of galanin with GnRH neurons, the intravenous administration of galanin has been reported to have no significant effect on LH release (10, 11). Moreover, the administration of galanin without GnRH coadministration could not stimulate the secretion of LH (28). The results of the current study taken together with the previously published findings may suggest that in goats galanin does not play a direct role in the secretion of gonadotropins at the pituitary level. It is possible that galanin decreases the release of GnRH from the median eminence, and thereby indirectly causes a decrease in pituitary secretion of LH and FSH.

An in vitro study in male calves demonstrated that galanin enhances the secretion of LH from pituitary tissue (29). However, in the current study, galanin was ineffective in modulating the release of gonadotropins in goats subjected to the 100% dietary energy intake. Age is one of the factors that has been reported to play a role in the modulation of hormone secretion by galanin. It has been suggested that galanin influences the secretion of gonadotropins during the perinatal and early life periods to a greater extent than in adult life (29). Galanin increases GH release from rat pituitary cells in the prenatal period but decreases the release of this hormone during adult life (29). Recently, it has been reported that in sheep the expression of galanin and galanin receptors increases from fetal to prepubertal age but decreases from prepubertal through to adult age (30). Therefore, it is possible that in ruminants the effect of galanin may be age-related and that galanin may play its role in the prepubertal period but not in adult life (30). The injection of $2 \mu g/kg$ BW galanin had no significant effects on the mean concentration of LH and FSH in all diets. This biphasic pharmacological effect has been reported for a number of different compounds. Biogenic amines influence the release of LH secretion in a dose-dependent manner. For example, dopamine at a low dose increases the secretion of LH whilst the opposite is true for a high dose of this compound (31). Furthermore, the dose-dependent effects of galanin have been observed in nociceptive reflexes, the acquisition and retention of which are all regulated by this peptide. One of the currently held beliefs is that such galanin-related biphasic effects are due to the existence of different galanin receptor subtypes. For example, Branchek et al. have shown that a high galanin dose inhibits the nociceptive reflex via the GALR1 receptor subtype whilst a low galanin dose causes hyperalgia via the GALR2 receptor subtype (3). Therefore, the results of the current study may suggest that a low galanin dose (1 µg/kg BW) increases the density of galanin receptors (GALR3 or other novel receptors), which are expressed in goats subjected to the higher energy diet (150%). However, these receptors did not respond in a similar fashion when a higher dose of galanin was administered in animals subjected to the same dietary restrictions.

In summary, galanin was shown not to markedly influence the secretion of gonadotropins in goats. Galanin may, however, reduce the mean plasma concentrations of LH and FSH in goats that are subjected to diets typified by higher energy intakes.

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