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SHORT NOTE [NOTA CORTA]

EFFECT OF EXOGENOUS GLUTAMATE SUPPLY ON THE ONSET OF PUBERTY IN GOATS II. SERUM LEVELS OF TRIIODOTHYRONINE

[EFECTO DEL SUMINISTRO DE GLUTAMATO EXÓGENO EN EL INICIO DE LA PUBERTAD EN CABRAS II. NIVELES SÉRICOS DE TRIIODOTIRONINA]

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SUMMARY

Thyroid hormones and their receptors in the ovaries are active regulators of reproductive function; both hyper- and hypo-thyroidism may result in estrous cycle disturbances. In addition, thyroid hormones elicit an extraordinary multiplicity of biochemical, cellular, and physiological responses, both in the simplest and the most complex organisms. On the other hand, glutamate, the main excitatory amino acid of the central nervous system has a marked stimulatory effect on the reproductive axis in mammals. In fact, occurrence of precocious puberty in response to administration of glutamate agonists has been reported in several species. The aim of the present study was to evaluate the effect of administration of glutamate on the onset of puberty in goats, and the association with serum triiodothyronine levels (T_3), as a possible metabolic signal for the onset of ovarian activity in juvenile goats. The study was carried out in northern Mexico (26˚ N) from June to October. Goats (n=18) were offered alfalfa hay (14% PC; 1.14 Mkal Kg^{-1} ENm), corn silage (8.1% PC, 1.62 ENm Mcal kg^{-1}), and ground corn grain (11.2% PC, 2.38 ENm Mcal kg^{-1}) under natural photoperiod. Location, animals, treatment design, preparation of the glutamate buffer solution, blood sampling scheme and quantification of serum P4 were described in the first part of this study. Serum samples were also evaluated for their content of T_3 by RIA. Final averages for live weight (LW) and body condition score (BCS) did not differ ($P>0.05$) between the Glutamate-supplemented and control groups (23.7±0.72 vs. 22.7±0.72 kg) and (3.69±0.10 vs. 3.38±0.10 units), respectively. The overall average for T_3 during the study was 1.47 ng mL^{-1}, with higher levels ($P<0.05$) in the glutamate-treated-goats (1.55 vs. 1.39±0.04 ng mL^{-1}). Results provide evidence that glutamate administration accelerated onset of puberty and that the onset of ovarian activity was positively related to high levels of serum T_3. These data support the hypothesis that goats are able to transduce exogenous glutamate infusions into cues for sexual maturation of juvenile animals, and that T_3 could be considered a metabolic modulator of the process leading to puberty in goats.

Key words: Goats, Glutamate, Puberty, Triiodothyronine, Progesterone.

INTRODUCTION

In mammals, initiation of puberty requires an increase in pulsatile release of GnRH from the hypothalamus; this increase is brought about by coordinated changes in transsynaptic and glial-neuronal communication. As the neuronal and glial excitatory inputs to the GnRH neuronal network increase, the transsynaptic inhibitory tone decreases, leading to the pubertal activation of GnRH secretion. The excitatory neuronal systems most prevalently involved in this process use glutamate and the peptide kisspeptin for neurotransmission/neuromodulation, whereas the most important inhibitory inputs are provided by γ-aminobutyric acid (GABA)ergic and opiatergic neurons (Terasawa and Fernández, 2001; Ojeda, 2006).

Glutamate and its receptors are involved in the maturation and maintenance of the neural mechanisms governing the preovulatory LH surge of young, reproductive-aged rodents and nonhuman primates (Jennes, 2002; Neal-Perry et al., 2005). Maturation of the GnRH neuronal network during the pubertal transition and hypothalamic control of estrous cyclicity are thought to involve changes in the balance

between excitatory input from glutamatergic and inhibitory input from GABAergic neuronal processes (Claypool et al., 2000).

Thyroid hormones are key regulators of metabolism and development and are known to have pleiotropic effects in many different organs (Boelaert and Franklin, 2005). Thyroid hormone elicits an extraordinary multiplicity of biochemical, cellular, and physiological responses in the simplest to the most complex organisms. The diverse actions of the biologically active thyroid hormone, L-triiodothyronine (T_3), can be divided into two aspects: growth and development, and regulation of metabolism (Tata, 2007). Although thyroid hormones are linked in many aspects to the reproduction process, their role in reproductive aspects such as puberty has not been widely studied. The aim of the present study was to evaluate the effect of administration of excitatory amino acids on onset of puberty, and their relationship with serum levels of triiodothyronine (T3), in prepuberal goats.

MATERIALS AND METHODS

The study was carried out at the Southern Goat Research Unit, URUZA-UACH, in northern Mexico (25° N, 103° W), at 1,117 m. Experimental area, environmental conditions, animals, feeding, preparation of the L-glutamate infusion, experimental design, blood sampling schedule and quantification of serum progesterone analyses and criteria to detect puberty were mentioned in the first part of this study.

Quantification of serum triiodothyronine (T_3) and progesterone, and criteria for evaluating reproductive activity

Blood samples were assayed for T_3 and P_4 content, by RIA. Goats with two consecutive P_4 serum levels ≥ 1 ng mL^{-1} were considered as reproductively active, and consequently pubertal animals (Cushwa et al., 1992). The between and within CV values were 0.66%, and 6.98% respectively, with a detection limit of 0.1 ng mL^{-1}.

Statistical analysis

Both body weight (BW) and body condition score (BCS) were compared considering an ANOVA-CRD, serum T3 levels across time were determined by split-plot ANOVA for repeated measures, while percentage of goats depicting or not ovarian activity were compared with a chi-square test. All the analyses considered the procedures of SAS (1991).

RESULTS AND DISCUSSION

While the initial average for BW and BSC were 16.65±1.04 kg, and 3.31±0.12 units, respectively, no differences ($P>0.05$) between experimental groups were observed for these variables along the experimental period (Table 1). An earlier ($P<0.05$) onset of puberty as well as a greater percentage of goats depicting ovarian activity were observed in the AA group compared to the C group. The overall average for serum T_3 level during the experimental period was 1.47 ng mL^{-1}, with higher ($P<0.01$) levels in the glutamate treated goats.

The hypothesis that administration of exogenous glutamate accelerates the onset of puberty, and that such event is positively related to high serum T3 levels was corroborated with these findings. Glutamate is the most important excitatory neurotransmisor in hypothalamus (van den Pol et al., 1990), and play a significant role in the establishment of the preovulatory surge of GnRH (Cicero et al., 1988). Activation of the NMDA receptors stimulate the release of GnRH in prepubertal and puberal animals (Bourguignon et al., 1989; Donoso et al., 1990; Gore et al., 1994) and accelerate puberty in monkeys and rats (Gore et al., 1996), while the administration of blockers of the NMDA delayed puberty in rats (Urbanski et al., 1990).

Table 1. Least square means for body weight (BW, kg), body condition score (BCS, units), goats depicting ovarian activity (Puberty, %) and serum levels of T3 (ng mL^{-1}) in juvenile crossbred goats under natural photoperiod in northern Mexico.

Variable	Glutamate	Control	SE[1]
BW, kg	23.75	22.8	0.72
BCS, units	3.69	3.39	0.11
Puberty, %	70.00[a]	25.00[b]	0.94
T3, ng mL^{-1}	1.56[a]	1.39[b]	0.04

[ab] Means in the same row with different superscript differ ($P<0.05$).
[1] SE, standard error of least squares means

The steroid-independent decline in LH pulse frequency that occurs as day length increases is dependent on the presence of thyroid hormones, and thyroidectomy prevents this decline (Anderson et al., 2002). This dependence on thyroid hormones is similar to the thyroid hormone-induced suppression of LH pulse frequency, due to increased responsiveness to the estradiol negative feedback at the beginning of anestrous. The role of thyroid hormones in the steroid-independent annual LH rhythm has not been well studied, although results of studies suggest a possible role for thyroid hormones in this system (Moenter et al., 1991).

Triiodothyronine is involved in tissue differentiation and growth, and in the regulation of numerous body functions, mainly by affecting metabolic rate. Thyroid hormones have a role in the control of seasonal reproduction in sheep. However, there is very little evidence suggesting that thyroid hormones are part of the signaling pathway linking energy balance and reproduction (Blanche et al., 2006). At present, it appears that thyroid hormones exert their major action at nuclear level by regulating the level of mRNAs of specific genes, and isoforms of receptors of thyroid hormones (TRs) mediate hormonal effects at the tissue level. TRs are recognized as members of a large superfamily of transactivating proteins involved in the regulation of gene expression.

Recent studies have shown an unexpected degree of complexity in the nature of the association of the T_3 receptors and the DNA of target genes. They have pointed toward possible multiple interactions between the T_3-receptor complex and other proteins participating in the process of gene regulation. These insights have provided a solid base for understanding differences in the level of thyroid hormone effects from one tissue to another (Schwartz et al., 1993). The existence of functionally connected genes controlling the pubertal process is consistent with the concept that puberty is under genetic control, and that the genetic underpinnings of both normal and deranged puberty are polygenic rather than specified by a single gene (Ojeda et al., 2006). Although the organization of this network of genes is not yet established, its existence is consistent with the notion that the onset of puberty is a process determined by the contribution of several genes (Krewson et al., 2004), which activation could be influenced by thyroid hormones, specifically triiodothyronine (T_3). Our results suggest that T3 could be considered a metabolic, even genomic, modulator of the process leading to puberty in goats.

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