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Glutamate supply positively affects serum cholesterol concentrations without increases in total protein and urea around the onset of puberty in goats

C.A. Meza-Herrera[a,*,1], G. Calderón-Leyva[a], M.J. Soto-Sanchez[a], J.M. Serradilla[b], A. García-Martinez[b], M. Mellado[c], F.G. Veliz-Deras[c]

[a] Universidad Autónoma Chapingo, Unidad Regional Universitaria de Zonas Áridas, A.P. No. 8, Bermejillo, Durango, 35230, Mexico
[b] Universidad de Córdoba, Departamento de Producción Animal, Campus de Rabanales, Ctra. Madrid-Cádiz, km 396, Córdoba, 14071, Spain
[c] Universidad Autónoma Agraria Antonio Narro, Periférico Raúl López Sánchez & Carretera a Santa Fe, Torreón, Coahuila, 27054, Mexico

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ABSTRACT

Different neurotransmitter and neuromodulatory systems regulate synthesis and secretion of GnRH. Whereas the endocrine and neural systems are activated in response to the metabolic status and the circulating levels of specific blood metabolites, glutamate receptors have been reported at hepatic level. This study evaluated the possible effect of glutamate supplementation upon changes in serum concentrations across time for total protein (TP), urea (UR) and cholesterol (CL) around the onset of puberty in goats. Prepuberal female goats ($n=18$) were randomly assigned to: (1) excitatory amino acids group, GLUT, $n=10$; 16.52 ± 1.04 kg live weight (LW), 3.4 ± 0.12 body condition score (BCS) receiving an i.v. infusion of 7 mg kg^{-1} LW of L-glutamate, and (2) Control group, CONT, $n=8$; 16.1 ± 1.04 kg LW, 3.1 ± 0.12 BCS. General averages for LW (23.2 ± 0.72 kg), BCS (3.37 ± 0.10 units), serum TP (65.28 ± 2.46 mg dL^{-1}), UR (23.42 ± 0.95 mg dL^{-1}), CL (77.89 ± 1.10 mg dL^{-1}) as well as the serum levels for TP and UR across time did not differ ($P>0.05$) between treatments. However, while GLUT positively affected ($P<0.05$) both the onset (207 ± 9 *vs.* 225 ± 12 d) and the percentage (70 *vs.* 25%) of females showing puberty, a treatment × time interaction effect ($P<0.05$) was observed in the GLUT group, with increases in serum cholesterol, coincident with the onset of puberty. Therefore, in peripuberal glutamate supplemented goats, serum cholesterol profile could act as a metabolic modulator for the establishment of puberty, denoting also a potential role of glutamate as modulator of lipid metabolism.

1. Introduction

In order to maximize reproductive success, the neuroendocrine system must regulate internal mechanisms to align them with respect to changes in the external environment (Scaramuzzi et al., 2006, 2011; Meza-Herrera and Tena-Sempere, 2012). First, throughout endocrine,

* Corresponding author at: Galeana 585 Poniente, Colonia Centro, Lerdo, Durango, 35150, Mexico. Tel.: +52 (871) 445 2691; fax: +52 (872) 776 0043.
E-mail addresses: cmeza2020@hotmail.com, cmeza2000@gmail.com (C.A. Meza-Herrera).
[1] See www.researchgate.net/meza-herrera

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anatomical and physiological changes, thereafter, right through reproductive behavior. An important set of endocrinological messengers and neurotransmitters participate in such regulation (Perfito and Bentley, 2009). Nonetheless, the common initiator is GnRH whose pulsatile release pattern constitutes a key link to line up the perception of external environmental conditions with respect to the hypothalamic-hypophyseal-gonadal (HHG) axis function (Perfito and Bentley, 2009; Ebling, 2009; Meza-Herrera et al., 2010, 2011). The activity and functionality of this neuronal circuitry is controlled through different neurotransmitters; activation of the complex KiSS-1/kisspeptin/GPR54 system and increased glutamatergic neurotransmission are two excitatory events stimulating the onset of puberty (Maffuci and Gore, 2009; Perfito and Bentley, 2009; Meza-Herrera et al., 2010; Meza-Herrera, 2012; Meza-Herrera and Tena-Sempere, 2012).

Besides to its role as the main neurotransmitter within the CNS, glutamate has also been involved in the modulation of metabolic activity, gene expression and protein synthesis (Parent et al., 2005; Meza-Herrera, 2008, 2012; Maffuci and Gore, 2009). However, reactivation of GnRH neurons at puberty not only involves changes in the expression of defined hormones or neurotransmitters (Meza-Herrera, 2008, 2012), but also can be modulated by the concentration of endogenous metabolites (Blache et al., 2006). Certainly, protein and amino acid requirements are important for growth and puberty while significant when considered within the context of body fuel utilization (Heitmann and Bergman, 1980; Meza-Herrera et al., 2004, 2007; Scaramuzzi et al., 2006, 2011; Gonzalez-Bulnes et al., 2011). Dietary low protein levels were reported to promote a reduction in body weight, exerting an inhibitory effect upon synthesis and release of LH. Therefore, serum protein level could be a key modulator of the neuronal processes involved in the increased pulsatile release pattern of GnRH observed around the onset of puberty (Meza-Herrera et al., 2007; Meza-Herrera, 2008, 2012; Gonzalez-Bulnes et al., 2011).

In addition, cholesterol, precursor of steroidal hormones, plays a fundamental role in the steroidogenic pathway which is required to promote follicular growth and development at ovarian level, in order for ovulation to occur (Gimpl and Gehring-Burger, 2007; Meza-Herrera and Tena-Sempere, 2012). Previous studies of our group demonstrated that in peripuberal goats, glutamate acts as an important cue for sexual maturation in a glucose-independent pathway, while both T3 and insulin affected in a significant fashion the establishment of puberty in goats (Meza-Herrera et al., 2011). Nonetheless, information regarding the relative changes in blood analytes that may serve as metabolic cues reactivating the communication of the HHG axis and triggering the onset of puberty in goats, is scarce. Building on the previous study, we aimed to elucidate whether glutamate supplementation could be associated with changes in the profile of blood metabolites around the onset of puberty; the blood analytes evaluated were total protein (TP), blood urea nitrogen (UR) and total cholesterol (CL) considering to the prepuberal goat as animal model.

2. Material and methods

2.1. Location, environmental conditions, animals and feeding

The present study was conducted at the Southern Goat Research Unit, URUZA-UACH (26° NL, 103° WL, at 1117 m). Prepuberal crossbred female goats ($n = 18$; 3 mo. old, 7/8 Saanen-1/8 Criollo), were fed a diet to meet 100% of their nutritional requirements adjusted for live weight (LW, NRC, 1998), considering a moderate average daily gain of 50 g d^{-1} during the 150d-experimental period. Goats were fed twice daily alfalfa hay (14% PC; 1.14 Mcal kg^{-1} ENm) in the morning, while corn silage (8.1% PC, 1.62 ENm Mcal kg^{-1}), and corn grain (11.2% PC, 2.38 ENm Mcal kg^{-1}) in the afternoon, under natural photoperiod. During the experimental period, which included from early June to early November, goats had *ad libitum* access to water, shades and mineral salts. Both LW and body condition score (BCS) were recorded prior to feeding, every 30 d during the whole experimental period; BCS was determined by one experienced technician in all the animals by palpation of the goat transverse and vertical processes of the lumbar vertebrae (L2 through L5) on a five point scale (1 = emaciated, 5 = obese). All the methods used in this study were conducted in accordance to accepted international guidelines (FASS, 1999).

2.2. Experimental design, blood sampling, blood metabolites, progesterone quantification and puberty

In early June, goats ($n = 18$, 12.0 ± 1.2 weeks old) were randomly allocated to one of two experimental groups: (1). Excitatory amino acid (GLUT, $n = 10$; 16.52 ± 1.04 kg, 3.4 ± 0.12 BCS) and (2). Control, (CONT, $n = 8$; 16.1 ± 1.04 kg, 3.1 ± 0.12 BCS). The GLUT group received an intravenous infusion of 7 mg kg^{-1} LW of L-glutamate twice a week throughout the experimental period (June–November); the CONT group received saline infusion. A total of 4 g of L-glutamate (Sigma Chemicals, St. Louis, MO) were dissolved in 50 mL distilled water to get a final solution for glutamate concentration of 80 mg mL^{-1}. Schedules for blood sampling collection and determination of the onset of puberty had been previously outlined (Meza-Herrera et al., 2011); the main activities will be only briefly considered. In all goats, blood samples (10 mL) were collected by jugular venopuncture, prior to feeding and twice per week from mid-June to late October. Blood samples were collected in sterile vacuum tubes (Corvac, Kendall Health care, St. Louis, MO), allowed to clot at room temperature for 30 min, and then serum was separated by centrifugation ($1500 \times g$, 15 min), decanted and stored in duplicate in polypropylene microtubes at −20 °C until hormonal analysis. Blood serum progesterone concentrations were determined using components of a commercial solid-phase RIA kit (Diagnostic Products, Los Angeles, CA, USA) validated for ruminant serum (Schneider and Hallford, 1996). Intra- and inter-assay CV were 9.9% and 12.4%, respectively; average recovery was 94%. Goats with serum P_4 concentrations ≥ 1 ng mL^{-1} in two consecutive samples were considered indicative that ovulation