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The influence of ovarian factors on the somatostatin–growth hormone system during the postnatal growth and sexual development in lambs

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A B S T R A C T

The aim of the study was to elucidate the effects of ovarian hormones on somatostatin in the hypothalamic neurons and growth hormone (GH) secretion during the postnatal growth and development of sheep. The study was performed on 9-week-old (infantile) lambs that were ovary-intact (OVI) or ovariectomized (OVX) at 39 days of age, and on 16-week-old (juvenile) lambs that were OVI or OVX at 88 days of age. Hormones in neurons and somatotropic cells were assayed with immunohistochemistry and radioimmunoassay. Following ovariectomy, immunoreactive somatostatin was more abundant ($p < 0.05$) in the hypothalamus of infantile lambs, whereas in juvenile lambs it was more abundant ($p < 0.05$) in the periventricular nucleus but reduced ($p < 0.01$) in the median eminence. In contrast to somatostatin in the hypothalamus, the content of immunoreactive GH in the hypophysis was less in OVX infantile lambs, but greater in OVX juvenile lambs ($p < 0.05$). Basal blood serum concentrations of GH were greater ($p < 0.05$) in OVX infantile lambs, whereas in OVX juvenile lambs, mean and basal concentrations of GH and amplitude of GH pulses were less than in OVI lambs ($p < 0.05$). The postnatal increase in body weight was greatest in middle-late infancy ($p < 0.01$). The body weight did not differ ($p > 0.05$) between OVI and OVX lambs. In conclusion, ovarian factors may inhibit the GH secretion in infantile lambs but enhance the GH secretion in juvenile lambs. Transition to puberty, as related to the growth rate, appears to be due mainly to change in gonadal influence on the somatostatin neurosecretion. A stimulation of somatostatin output in the median eminence by gonadal factors in infancy is followed by a stimulation of somatostatin accumulation after infancy. Thus, ovarian factors modulate mechanisms within the somatotropic system of lambs to synchronize the somatic growth with sexual development.

1. Introduction

There is a close association between growth and reproductive development (Hull and Harvey, 2002; Veldhuis et al., 2006). In sheep, gonadal factors may play a stimulatory role in GH secretion until puberty (Wańkowska, 2012). Thus, transition to puberty depends upon an accelerated growth during infancy until weaning (Suttie et al., 1991; Wańkowska et al., 2008; Wańkowska, 2012) and following increase in gonadotropic hormones secretion (Wańkowska et al., 2010a,b). The interactions between somatotropic and gonadotropic factors after the neonatal period in female lambs are not well understood. Gonadal steroids enhance GH release in ewes (Dutour et al., 1997; Landefeld and Suttie, 1989; Scanlan and Skinner, 2002). It was, therefore, hypothesized that the postnatal development involved changes in the effect of ovarian hormones on GH secretion in lambs.

Episodic secretion of GH is generated by hypothalamic and hypophyseal mechanisms of interaction between

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hypothalamic GH-releasing hormone (GHRH) and the GH release-inhibiting peptide, somatostatin (Plotsky and Vale, 1985). Circulating GH inhibits its own secretion *via* feedback (repression) on GHRH and feed-forward (stimulation) on somatostatin (Farhy et al., 2002). It was demonstrated using a minimal biomathematical model that activation of the GH autofeedback drive of somatostatin outflow induced a female-like pattern of GH release (Farhy et al., 2002). Thus, it seems that this regulatory mechanism should be important for the ontogeny in female mammals. The sexually dimorphic pattern of GH secretion (for review see Farhy et al., 2002), which influences differences in male and female somatic growth, is determined by the somatostatin-producing neural center situated in the anterior periventricular area of the hypothalamus (Gabriel et al., 1989; Jansson et al., 1985). This population of neurons is not detectable until the last days of prenatal life in female lambs (Polkowska, 1986) it becomes prominent after the neonatal period (in early infancy), and appears to be fully developed by 8–10 weeks of age (*i.e.* in middle infancy) (Polkowska et al., 1987). From the periventricular nucleus (PEV) arises somatostatinergic innervation of the median eminence (Epelbaum et al., 1981; Kiss, 1997; Willoughby et al., 1995) and the neurons of this nucleus are the major source of somatostatin in the hypophyseal portal system (Bluet-Pajot et al., 1998). From these morphological findings, the development of physiological basis for somatostatin action in the adenohypophysis can be expected to be initiated during middle infancy in weanling lambs. However, the suppressive effect of somatostatin on the GH response to GHRH increases at birth (De Zegher et al., 1989a) and GH secretion in the neonate is more sensitive to the inhibitory influence of somatostatin than in the fetus (Gluckman, 1984; Torronteras et al., 1997). Reports concerning the secretory function of somatotropes during the accelerated growth in infantile lambs reveal that serum GH concentrations decrease in late infancy (at approximately 12 weeks of age) and no obvious pubertal increase occurs (Basset and Gluckman, 1986; Suttie et al., 1991; Wańkowska et al., 2008). The mechanisms of the decrease in circulating GH during infancy in sheep are poorly described. However, it seems that the regulation of the postnatal growth may be related to increasing negative feedback within the somatotropic axis mediated *via* increased somatostatin release (Spencer et al., 1983; Wańkowska et al., 2008).

The mechanism underlying activation of the GH negative autofeedback loop in female lambs has yet to be determined. Thus, the objective of this study was to use ovariectomy and histomorphological and functional criteria to investigate the influence of ovarian factors on the development of the somatostatin–GH system as related to the rate of somatic growth in infantile and juvenile lambs.

2. Materials and methods

2.1. Animals and management

All procedures were conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki), the EU Directive 2010/63/EU for animal experiments as approved by the Local Ethics Committee affiliated with Warsaw Agriculture University (number of opinion 29/2006), according to the Polish Law for the Care and Use of Animals (2 August 1997).

The 3- to 4-year-old Polish Longwool ewes were mated naturally at a commercial sheep facility (Samoklęski Farm, Poland). The ewes were transported to the Institute of Animal Physiology and Nutrition (Jabłonna, Poland; 52°N latitude and 21°E longitude) in September. Sixteen female lambs from twin pregnancies and 4 from singleton pregnancies were born in February. The lambs were penned indoors with dams, in individual pens, under natural conditions of light and temperature. The ewes were fed a diet of commercial concentrates, with hay and water available *ad libitum*, that provided 100% of the nutritional requirements recommended by the National Research Institute of Animal Production for pregnancy and lactation in sheep (Instytut Zootechniki, 1993). Ten lambs were weaned on the 65th day of age. They were fed hay *ad libitum* and a complete pelleted concentrate ration supplemented with vitamins and minerals, and containing 17% of protein twice daily. This diet assured the optimal growth rate defined by the changes in the slope of the curve of body weight gain, which was evaluated by weighing at 1, 39, 63, 88 and 112 days of age (Fig. 1).

2.2. Experimental design

Four-week-old lambs were divided randomly into 4 groups ($n = 5$ per group) according to two phases of ontogeny, infancy and the beginning of the juvenile period. These periods had been determined on the basis of intrahypophyseal regulation of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and GH secretion (Wańkowska et al., 2008, 2010a,b; Wańkowska and Polkowska, 2006), and development of the digestive tract. The investigation was performed in 9-week-old weanlings (63 days of age; middle infancy) that were ovary-intact (OVI) or ovariectomized (OVX) at 39 days of age (early infancy). They suckled their mothers 3–4 times per 24 h. The second experimental group consisted of 16-week-old lambs (112 days of age; early juvenile period) that were OVI or OVX at 88 days of age (late infancy).

A day before collecting blood plasma, a jugular venous catheter was inserted and filled with heparinized saline. Blood samples were collected every 10 min over a 5-h period (from 08:30 am to 01:20 pm) in 5- (37 days of age), 9- (63 days of age), 12- (86 days of age) and 16-week-old (112 days of age) OVI lambs and 9- and 16-week-old OVX lambs. During collection, the lambs were kept in pens where they could lie down and had unlimited access to hay, water and/or dams. Blood samples were centrifuged in heparinized tubes and the plasma was stored at −20 °C. On the day of hypothalami and hypophyses collection, the 9-week-old and 16-week-old OVI and OVX lambs were anesthetized with an intravenous injection of pentobarbitone sodium (20 mg/kg; Biochemie GmbH, Kundl, Austria) and decapitated (from 01:30 pm to 02:30 pm).