Stress and nutritional influences on GnRH and somatostatin neuronal systems in the ewe

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Abstract. The effect of stressful stimulation and protein malnutrition on the gonadotrophic and somatotrophic axis of sheep is discussed with special references to the relationship between these stimuli and the GnRH and somatostatin neuronal systems in the hypothalamus. Generally, long-term stimulation and chronic underfeeding reduce gonadal functions in the sheep. There is evidence for the GnRH-dependent pathway for the mechanism of these phenomena in female sheep. GnRH neurons respond to long-term stress in diminishing of neuropeptide release from the nerve terminals due to the depression of its axonal transport. Chronic restriction of dietary proteins in lambs reduces the plasma LH concentrations but does not impair the development of GnRH neurons nor the synthesis and processing of GnRH. It is suggested that malnutrition delays the first ovulation probably due to the neural mechanism responsible for the preovulatory GnRH/LH output. Stress has rather unclear effect on growth hormone (GH) secretion in the sheep. Prolonged, but not short stressful stimulation provokes the rapid release of somatostatin, which is sustained during long-term stimulation. These results suggest that effect of stress on somatotrophic axis depends on the period of stressful stimulation. Chronic malnutrition enhances secretion of GH by an increase in amplitude of GH pulses and reduces the secretory activity of somatostatin neurons. It is postulated that nutrients can influence GH secretion in the sheep by mechanism dependent on the hypothalamic somatostatin.

Key words: footshocks, malnutrition, hypothalamus, GnRH, somatostatin, ewe
INTRODUCTION

Cumulative evidence supports the view that two environmental factors, namely stressful stimulation and a low level of nutrition modulate growth and affect reproduction of animals. Although the precise nature of these interactions is complex and often unclear, many appear to act via hormonal mechanisms. The endocrine responses to these environmental cues generally begins on the level of the central nervous system (CNS). There is strong evidence for the existence of hypothalamic dependent pathways for this mechanism. In the first place the hypothalamic neuropeptides responsible for the growth and/or reproduction are activated. Next, these changes modulate the secretion of related pituitary hormones and finally provoke physiological responses such as the restriction of body mass, delay of puberty or disturbances in the course of the estrous cycles and ovulation. Generally in mammals stress and low planes of nutrition have a restrictive effect on GnRH/LH release, but an inverse effect can occur on growth hormone secretion in different mammalian species. The hormonal response to the noxious stimuli may vary according to the nature of stressor and the length of time in which stressor is applied. The problem in the duration of stressful stimulation is rather complicated because the hormonal responses for short, prolonged or chronic stimulation differ considerably (Polkowska and Przekop 1993). Also the various dietary components may cause different hormonal responses (Feifel and Vaccarino 1994). Changes in the gonadotrophin secretion can be provoked only by intense or prolonged signals (Rivest and River 1995). Thus, the relationship between noxious environmental cues and the endocrine response of an organism riddled with complexity. This review will focus solely on the associations between electrical stressing and/or nutritional factors and the morphological activity of hypothalamic neuronal systems, producing GnRH and somatostatin, with references to related pituitary cells. Before describing the influence of noxious stimuli on these neurons it would be instructive to summarize briefly the anatomical organization of these peptidergic systems in the sheep brain.

GnRH- NEURONAL SYSTEM

Anatomical organization

The location and characterization of neuronal structures containing GnRH in the sheep has been described widely by Polkowska et al. (1980), Lehman et al. (1986) and Caldani et al. (1988). As in other mammals, notably in rodents, GnRH neurons in the sheep are not attributed to any special nuclei but are scattered widely from the rostral septum, area preoptica, anterior hypothalamic area and medial basal hypothalamus (MBH). The number and morphology of perikarya, axons and nerve terminals strongly depend on the age and the physiological state of an animal (Polkowska et al. 1980,

![Fig. 1. Organization of GnRH neurons in the sheep brain; a, immunoreactive GnRH perikarya and fibers in the area preoptica, x224; b, distribution of immunoreactive GnRH in the nerve terminals of the central part of the median eminence, x28.](image)
Lehman et al. 1986). In the anestral or injected with colchicine adult ewes about 1,200-2,500 perikarya per brain have been found (Lehman et al. 1986, Caldani et al. 1988). About 95% of the GnRH-cells are located in the preoptico-hypothalamic region of the brain and 50% in the area surrounding the organum vasculosum of the lamina terminalis (OVLT) (Fig.1a). Very little GnRH-cells (15%) has been found in the MBH (Caldani et al. 1988). The majority of neurons originating from these perikarya are projected in the direction of the OVLT and the median eminence (ME). Only 5% was found in other formations of the brain (Caldani et al. 1980). Neurons are projected to the ME along at least two routes: a major ventrolateral projection above the optic tract and the less prominent pathway along the third ventricle (Lehman et al. 1986). The immunoreactive (ir)GnRH terminals in the ME extend in a dense network at the ventral edge of its rostral section. In the central region, the densest concentration of the terminals are located in lateral parts of the external layer in the immediate proximity of the capillaries located at the junction of the ME and the pars tuberalis of the pituitary gland (Fig.1b).

**Stressful stimulation (footshocks)**

In the ewes stressful stimuli generally reduce gonadal functions. Premating stress delays the onset of the estrus and lowers the ovulation rate (Domay et al. 1973). Psychoemotional state evoked by prolonged intermittent weak electric stimulation of cycling ewes causes long lasting disturbances in the course of the estrous cycles. These disturbances are revealed in the functional and structural changes in the ovaries such as cystic degeneration of graafian follicles or inactive ovaries. These animals have exhibited no preovulatory release of LH at the expected time of estrous (Przekop et al. 1984). In ovariectomized sheep, confinement stress affects episodic secretion of LH (Rasmussen and Malven 1983). The data obtained mostly from laboratory animals proves that gonadal atrophy and reduced sexual activity caused by chronic stress, primarily is due to interference with the central regulation of the pituitary gonadotrophin secretion (reviewed by Rasmussen and Malven 1983). Data supporting this view, especially concerning domestic animals is limited. Also there is no data about the effect of environmental cues on neurons producing hypophysiotrophic hormones. In our experiment performed on sheep, the immunocytochemical technique was applied to monitor of the eventual changes in the secretion of peptide on the levels of their synthesis, transport and release to the blood. Female sheep were stimulated with electrical footshocks through short (4 h), prolonged (3 days) and long-term (12 days) period, in anestrous season, when the GnRH neuronal system is "quiet" (Polkowska and Przekop 1988, 1992, Przekop et al. 1988). After short stimulation no changes were observed in the morphology of GnRH neurons. From the third day of the stimu-
TABLE I

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>median area</th>
<th>area</th>
<th>medial basal</th>
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<tr>
<td>Control (5 animals)</td>
<td>1640 ± 162 21.5 ± 6.4 102.8 ± 24.3</td>
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</tr>
<tr>
<td>Stressed (5 animals)</td>
<td>2395 ± 280a 40.5 ± 7.9a 46.0 ± 10.5a</td>
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**Significantly different at P<0.001; Adapted from Przepk et al. 1988**

ation, some alternations in GnRH neurons activity, even more pronounced after long-term (up to 12 days) stress was witnessed. The pattern of these changes comprised elevation of the GnRH concentrations in the ME, the gradual increase in density of ir GnRH material in the ME, increase in the number of GnRH perikarya in the area preoptica as well concentrations of GnRH in this part of the brain (Fig. 2ab, Table I). The morphology of the pituitary LH cells changed very slowly during the stressful procedure. Significant increase in their number with concomitant increase of ir LH material in cell plasma was noticed only after 12 days of stimulation. The daily mean LH concentration in blood plasma and LH pulse frequency displayed a similar variation in all intervals of stimulation compared with unstressed animals (Polkowska and Przekop 1992). This experiment revealed evidence that long electrical stressful procedure in anestrous ewes provokes changes in the morphological activity of the GnRH neuronal system. It is suggested that some mechanisms connected with the release of GnRH from the ME and with its transport along neuronal fibers within the hypothalamus, but not with its synthesis, are impaired.

**Nutritional factors**

It is generally accepted that nutrition modulates reproductive endocrine functions in many species including sheep (reviewed in Lindsay et al. 1993). In growing female sheep lowered planes of nutrition delay puberty (Foster and Olster 1985) through the depression in frequency of LH pulses which are vital to follicular development (Foster et al. 1989). In male sheep severe malnutrition changes testicular growth and endocrine functions of testes (Martin and Walkden-Brown 1995). The mechanisms responsible for these changes is not yet defined. It is postulated that there are at least two ways to trigger these endocrine functions. First, GnRH-dependent pathway, when nutrients supply changes in GnRH pulse frequency and thus LH/FSH secretion (Tatman et al. 1990, Kile et al. 1991). Second, strongly supported by the data obtained from males (Martin and Walkden-Brown 1995), GnRH independent way, when nutrients act directly upon testicular tissue changing the production of hormones and germ cells. In females, nutritional retardation of sexual maturation is initiated by disturbances in GnRH secretion, but it is still not known whether the synthesis or the release of the hormone that is impaired during restricted feeding (Ebling et al. 1990, Tatman et al. 1990, Kile et al. 1991). To test this question, female lambs 3 month old were fed during 5 month on a diet restricted up to 8% of protein (Polkowska and Przekop 1993). These ewes did not reach sexual maturity at the same time as the animals fed on standard diet. The examination of GnRH neurons revealed that all elements of this system were normally developed. Gonadotropin-associated peptide (GAP) of the GnRH precursor was present in the same population of neurons that contained GnRH in the sheep brain. Some visible fluctuations were observed in the feature of LH-pituitary cells, which were hypertrophied, three times more numerous than in standard fed group, and contained a high concentration of the ir-material. This kind of morphological change suggest a diminishing release of the hormone from the cells and this suggestion can be correlated with significantly lower concentrations of plasma LH measured in the middle of the experiment. The data presented give evidences that a restriction of proteins in the diet of growing female sheep delays their puberty but does not impair
the synthesis and processing of GnRH in the brain neurons and synthesis of LH in pituitary cells. It is suggested that retardation in sexual maturation as a result of protein restriction can be caused by an impairment of the release of GnRH rather than by changes in its synthesis and processing.

**SOMATOSTATIN NEURONAL SYSTEM**

**Anatomical organization**

The somatostatin hypothalamic neuronal system in adult sheep reveals a common pattern of an organization when compared to many mammalian species. Unlike to the localization of GnRH, whose perikarya are scattered among different brain nuclei, somatostatin perikarya create a relatively compact center situated in the periventricular region of the anterior hypothalamus (Fig. 3a). This region covers partly the suprachiasmatic nucleus and paraventricular nucleus (Polkowska et al. 1987, Tillet et al. 1989). An additional population of so-called "shell perikarya" has been recently found around the nucleus ventromedialis and in posterior part of the nucleus arcuatus (Willoughby et al. 1995). In this region of the MBH, only a distinct concentration of somatostatin fibers without cells has been found in intact growing lambs (Polkowska et al. 1987). In the rat, at least 70% of somatostatin neurons from the periventricular region but not those in MBH are projected to the ME and form a final common pathway for the regulation of GH secretion in the anterior pituitary (Merchenthaler et al. 1989). The somatostatin nerve terminals in the sheep ME are extremely abundant. They occupy the entire width of the external area from palisade layer to the perivascular zone throughout the central to caudal extent of the ME and the pituitary stalk (Fig. 3b). It should be mentioned here that somatostatin perikarya are spread in many extrahypothalamic formations. It is believed that they are independent of the hypothalamic system and that somatostatin produced by these neurons acts as neurotransmitter rather than a hypophysiotrophic hormone (Krisch 1980).

**Stressful stimulation (footshocks)**

It is generally accepted that prolonged or chronic stress causes disorders in GH secretion, which plays crucial role in the body metabolic activity. Stressful stimuli alter the pattern of GH secretion in a different manner in various species. In the rat, both acute and chronic stressful stimulation and also different kinds of stressors, all suppress pulsatile secretion of GH from the pituitary gland (Lenox et al. 1979) and this suppression can be mediated by the hypothalamic somatostatin (Terry et al. 1976). In contrast, various stressful stimuli elicit an increase of circulating GH in monkeys and humans (Schalch 1967, Brown et al. 1971). The data concerning GH secretion under noxious stimuli in ruminants are limited.
and heterogenous. In cows GH responds in specific manner to different kinds of stressful stimuli (Munksgaard and Levendahl 1993), in the sheep cold exposure does not change GH secretion (Christensen et al. 1990). In our experiment performed on anestrous sheep, the influence of short (20 min), prolonged (3 days) and long-terms (8 and 12 days) intermittent electrical pulses on hypothalamo-pituitary somatotrophic axis was tested. An additional group of animals was stimulated for 12 days and then subjected to 12 days recovery period (Polkowska and Przekop 1988, Polkowska 1989). The short stimulation did not elicit any morphological changes in the somatostatin neurons, although it immediately activated the CRF neurons (Polkowska and Przekop 1988). Prolonged footshocking induced a sharp depletion of the ir somatostatin material stored in the ME (Fig. 4a, b) and pituitary stalk and a marked diminishing of the staining intensity of the somatostatin perikarya. These kind of changes were maintained in hypothalami of long-term stressed animals, but after 12 days recovery period, somatostatin content in the ME and the feature of somatostatin perikarya returned to the control values (Polkowska 1989). In all periods of the stimulation no changes in the number and morphology of the GH-cells were observed. However in the tissue from the last group, the percentage of GH-cells decreased almost 50% as compared with controls. (Polkowska 1989). We have no data concerning GH plasma levels in this experiment. Recently, Przekop et al. (1995) reported an increase in daily levels of plasma GH in sheep during the first day of footshocks stimulation, with a tendency to decrease over the next two days of stimulation. However during long-term stimulation up to 12 days no changes in GH concentrations were noticed (personal communication). The presented immunocytochemical results describing only neuronal activity of somatostatin in the hypothalami of stressed sheep are insufficient for a precise explanation of this phenomenon, because there is still a lack of study about the effect of stress on the second factor influencing GH secretion-growth hormone releasing hormone (GHRH) in this species. However the presented work does provide the first evidence of the release of somatostatin in conditions of prolonged stressful stimulation. It seems that this release is maintained during long-term stressing, although it has no visible effect on the GH-cells. The cessation of somatostatin release during recovery period can be responsible for the depletion of GH granules from the pituitary cells which is manifested by their sharp decrease in number.

**Nutritional factors**

The disturbances in the supply of nutrients reach the somatotrophic axis, which play an essential role in the coordination of interactions, leading to somatic growth. Changes in GH secretion induced by malnutrition vary with the species. In rats the con-
centration of blood plasma GH is reduced under conditions of restricted feeding (Tannenbaum et al. 1979). In ruminants, rabbits and humans the effect is opposite (e.g. Hart et al. 1985, Foster et al. 1989). In adult ewes this kind of response is associated with the increased synthesis as well as release of GH from the pituitary gland (Thomas et al. 1990). The mechanism by which the intake of nutrients alters GH is unknown. There is evidence, that in sheep the major role in the control of GH secretion is played by GHRH (Magnan et al. 1995), but in conditions of limited feeding, the role of GHRH in this process is less important. Exogenous GHRH does not change the concentration of plasma GH in sheep feed the diet limited in energy (Hart et al. 1985). Also restricted feeding does not affect secretion of hypothalamic GHRH (Thomas et al. 1991). It is supposed that dominant role in the control of GH secretion in underfed sheep is played by somatostatin. This concept is suggested by the observation that

Fig. 5. Effect of chronic protein deficiency in the diet on the somatotrophic axis of lambs; a, immunoreactive GH cells in the pituitary gland of lamb fed standard diet, x 70; b, immunoreactive GH cells in the pituitary gland of lamb fed low protein diet, x70; Note the increased number of GH cells in the lamb fed a low protein diet; c, immunoreactive somatostatin material in the median eminence of lamb fed standard diet, x28; d, immunoreactive somatostatin material in the median eminence of lamb fed low protein diet, x28, ri-recessus infundibularis. Note the conspicuous diminishing of the somatostatin stores in the lamb fed low protein diet; e, immunoreactive somatostatin perikarya in the nucleus paraventricularis of lamb fed standard diet, x28; f, immunoreactive somatostatin perikarya in the nucleus paraventricularis of lamb fed low protein diet, x28. Note the increased number and stain intensity of cells in the lamb fed a low protein diet. v, third ventricle of the brain.
changes in peripheral blood GH characteristics in female lambs fed 14 weeks standard or low protein diet. Blood samples were collected from animals during 4 h with 15 min interval. Data were calculated using Pulsar analysis, and the mean concentration of GH for each animal was calculated from the area under the curve. Data are presented as mean values with standard deviation, n = 7.

<table>
<thead>
<tr>
<th>Diet</th>
<th>% of protein</th>
<th>14.2</th>
<th>8.1</th>
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<tbody>
<tr>
<td>Mean GH</td>
<td>ng/ml</td>
<td>1.48 ± 0.44</td>
<td>3.68 ± 1.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of pulses</td>
<td>n/240 min</td>
<td>2.86 ± 0.69</td>
<td>3.86 ± 1.07</td>
</tr>
<tr>
<td>pulse amplitude</td>
<td>ng/ml</td>
<td>1.31 ± 0.68</td>
<td>4.54 ± 3.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Inter-pulse interval min</td>
<td></td>
<td>90.0 ± 35.4</td>
<td>67.0 ± 18.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup>Significantly different at P<0.05; Adapted from Polkowska et al. 1996.

During restricted feeding the concentration of somatostatin in portal blood is reduced by half (Thomas et al. 1991). This data implies that the GH response to a restricted diet can be directly associated with changes in the activity of the somatostatin hypothalamic neuronal system. This hypothesis was then tested in our laboratory (Polkowska et al. 1996). The experiment was performed on growing lambs fed isocaloric diets containing standard (14%) or lowered content of proteins (8%). The results showed that after three and four month of experimental feeding, the mean concentration of GH was significantly higher in the group fed a low protein diet and this difference was due to significant elevation of GH pulse amplitude (Table II). Prolongation of restricted feeding up to 5 month resulted in conspicuous increase of GH cells number in the pituitary gland (Fig. 5a, b). The ir somatostatin stores in the ME of these animals markedly dropped out but the concentration of the ir material in the somatostatin perikarya increased (Fig. 5c-f). This can be interpreted as a disturbance in axonal transport of peptide from the perikarya to the nerve terminals (Polkowska et al. 1996). These results indicate that an increase of the GH secretion due to elevation of its amplitude of pulses in the sheep fed of restricted in proteins diets can be elicited by changes in the somatostatin neurons secretory activity. It is suggested that chronic malnutrition augment GH secretion in growing lambs as a result of a decrease in the hypothalamic somatostatin output due to the suppression of its axonal transport.

**CONCLUSIONS**

From the preceding discussion it is proposed that both stimuli, long-term stressing and chronic protein undernutrition elicit in sheep the same overall pattern of endocrine responses in gonadotrophic and somatotrophic axes which begins at the level of the CNS. In the gonadotrophic axis both challenges evoke a restraining effect on the GnRH neuronal system, which leads to changes in the secretion pattern of this peptide, and in consequence to some disturbances in the estrus cycle. The action of both stressful signals on the somatotrophic axis seems to be more complicated. They affect GH secretion via changes in somatostatin neuronal system activity, but their action has an inverse effect: stress stimulates somatostatin release; undernutrition restrains its output.

**REFERENCES**

Central mechanism controlling pulsatile luteinizing hormone secretion in the nutritionally growth-limited female lamb. Endocrinology 126: 2719-2727.


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