

Effects of central infusions of neuropeptide Y on the somatotrophic axis in sheep fed on two levels of protein

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Abstract. Effects of infusions of neuropeptide Y (NPY) into 3rd ventricle of growing sheep fed on diets containing restricted (R) or elevated (E) levels of protein on the immunoreactive (ir) somatostatin neurones, ir somatotrophs, growth hormone (GH) concentration in the blood plasma were studied. The long-term restriction of protein in the diet elicited: enhancing irSS content in periventricular perikarya; diminishing irSS stores in the median eminence and elevating the number ir somatotrophs and content of irGH. NPY infusions enhanced the content of irSS in perikarya in sheep fed on E diet and diminished the number of ir somatotrophs and content of irGH of sheep fed on R diet. The R diet as well as NPY infusions caused an increase in GH mean concentrations in the blood plasma. Obtained results suggest that stimulatory effect of restricted feeding and/or NPY action on GH secretion can be due to attenuated SS output. Since dietary restrictions and exogenous NPY have similar influence on the activation of GH secretion, we suggest that NPY could be a neuromodulatory link between nutritional cues and somatotrophic axis in sheep.

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INTRODUCTION

Nutrition is one of the environmental cues that affects the somatic growth of ruminants. The secretion of growth hormone (GH), the principal hormone of the somatotrophic axis coordinating postnatal growth, is sensitive to dietary restrictions in the sheep. Chronic restrictions of protein in the diet of growing female lambs augments mean plasma concentration of GH that is associated with significant elevation of its pulse amplitude (Polkowska et al. 1996). Chronic limitation of food energy intake increases the synthesis and release of GH in adult ovariectomized sheep (Thomas et al. 1990). The neuroendocrine mechanism by which intake of nutrients alters hypophysial GH secretion is poorly known. In ruminants, GH changes during restricted feeding appear to be directly associated with the role of somatostatin (SS). In ovariectomized ewes, long-term dietary restrictions result in decreased release of SS into hypophysial portal vessels without effect on growth hormone releasing hormone secretion (Thomas et al. 1991). The response of insulin like growth factor-1 (IGF-I) on dietary manipulations, especially in long-term conditions, is less known. IGF-I mediates the anabolic actions of GH in peripheral tissues (Daughaday and Rotwein 1989) and appears to be an important factor in the regulation of protein metabolism (McGuire et al. 1992). This factor is believed to be a good hormonal indicator of nutritional status of an organism in many species (Jahreis 1993). Neuropeptide Y (NPY), a powerful central appetite stimulator, seems to play an integrative role between appetite regulation system and endocrine axis (Morley 1987). It has been shown that NPY can be a neuromodulatory link between feeding and reproduction in sheep (McShane et al. 1992, Ober and Malven 1992). Moreover, its integrative role between nutrients and the somatotrophic axis has been shown only in rats (Rettori et al. 1990; Catzefflis et al. 1993, Pierroz et al. 1995). In this species central administration of NPY causes dose-dependent inhibition of GH release from the pituitary cells and a decrease of GH and IGF-I concentrations in blood plasma (Catzefflis et al. 1993, Pierroz et al. 1995). Thus, in this species, NPY has an inhibitory effect on GH release by stimulation of SS (Rettori et al. 1990). It has been shown that central infusions of NPY modulate food intake in many species including sheep (Miner et al. 1989), but its effect on GH release in this species is not known. The dietary effects on body mass and GH secretion in the sheep may be mediated by the NPY

hypothalamic neuronal system, since increase of NPY immunoreactivity in the hypothalamus and enhancement of the levels of NPY mRNA in the arcuate nucleus and the median eminence (ME) has been observed in the conditions of restricted feeding in the ovariectomized sheep (McShane et al. 1993, Barker-Gibb and Clarke 1996).

The aim of the present study was to investigate the hypothesis that NPY can mediate interactions between nutritional factors like protein content and the hormones of the hypothalamo-pituitary-somatotropic axis in female sheep. Since dietary protein restrictions result in increased GH secretion via decrease of SS secretory activity and in elevated levels of NPY in the brain, it is likely that NPY may be involved in the mechanism by which malnutrition affects somatotrophic function. In the present study we sought to determine whether the administration of NPY to the 3rd ventricle of the brain affects SS immunoreactivity in neurones in the hypothalamus, immunoreactive (ir) somatotrophs in the pituitary gland and GH and IGF-I profiles in the blood plasma in a similar manner to that described for feeding with insufficient doses of protein.

METHODS

Animals

Twenty lambs [(Merino x Romanov) x Suffolk] born in the first two weeks of March at 6 months of age and similar weight (30 kg) were put into individual pens. They were placed into two groups, one of which was fed a diet supplemented in protein by 9% above the recommended level (E) and the other restricted by 48% (R), to the end of the experiment (17 weeks - experimental sheep and/or 15 weeks - eight control sheep for immunohistochemistry - IHC). Both diets were equal at the level of 5% of body weight daily, composition of minerals and vitamins was the same and net energy value was equal to 5 MJ/kg dry matter. Composition of diet E (18% of protein) was: meadow hay 33.1%; oat straw 6.5%; dried sugar beet pulp 35% and soybean 25.2% as the main source of protein. Diet R (8.1% of protein) was: meadow hay 6.5%; oat straw 33.3% (instead of soybean); dried sugar pulp 60%. Daily rations were divided into 2 equal feeds given at about 08.00 and 14.00 h. The daily food allowance was 9.8% of the metabolic body weight $BW - kg^{0.75}$ adjusted every 2 weeks for the actual body weight. The animals were weighed every two weeks and regularly inspected by a veterinarian. In the

12th week of the experimental feeding sheep were subjected to the surgical procedure.

Experimental procedure

Implantation of cannulae was performed at least three weeks prior to the first infusion. Animals were s.c. injected with atropine (Polfa, Warsaw, Poland) at a dose of 0.05 mg/kg of body weight. Fifteen min later pentobarbital ("Vetbutal" Biowet, Pulawy, Poland) was given i. v. at a dose of 10–20 mg/kg of body weight. Animals under deep anaesthesia were fixed in a stereotaxic apparatus using the procedure of Traczyk and Przekop (1963). Briefly, permanent stainless-steel guide cannulae (0.6 mm) were lowered towards the 3rd ventricle of the brain (position antero-posterior 31 mm, lateral 0.5 mm along sagittal plane 0.10) using the stereotaxic coordinate system of Welento et al. (1969). The cannulae were secured to the skull with dental cement and screws. Each guide tube was fitted with an indwelling stilette to prevent a backflow of the cerebrospinal fluid. The correct placement of the guide cannula was established by the efflux of cerebral spinal fluid upon removal of the guide tube stilette after the surgery and before each infusion. Additionally, the placement of the guide cannulae was checked by inspections of brains after decapitation. After the surgery, penicillin-streptomycin (Scan-Vet Poland) 0.04 ml/kg of body weight was given s.c. during 4 consecutive days. One day before each infusion a jugular venous catheter was inserted into each sheep and kept with heparinized saline (50 units heparin/ml in 0.9% w/v NaCl).

Immediately before each infusion, the stilette was removed from the guide cannula and an injector needle connected to a length of tubing (Hythe, Kent, UK) containing the solution to be injected was lowered to a depth approximately 2.5 mm above the base of the brain at the level of the medial basal hypothalamus. The infusions were performed at a flow rate of 40 μ l/min using CMA 100 microinjection pump (CMA/Microdialysis AB, Stockholm, Sweden) for 5 min. Infusions were given at 12.00 h each day. Six animals from E and six from R groups were infused intracerebroventricularly (icv) in one week intervals with Ringer solution, 5 μ g NPY and 50 μ g NPY (Sigma) dissolved in 200 μ l of Ringer solution, according to McShane et al. (1992). Four animals from each nutritional group were infused icv with only Ringer solution, and these sheep constituted the control group for IHC estimations. Blood samples were drawn on the day of the infusions at 10 min intervals over the 6 h

period between 09.00 and 15.00 h. Samples were centrifuged and collected plasma was stored at -20°C until analysis. Sheep were slaughtered 3 h after the last infusion by decapitation under pentobarbital anaesthesia in the local licensed slaughterhouse.

All procedures during the experiment were approved by the Ethics Committee at the Kielanowski Institute of Animal Physiology and Nutrition in Jabłonna, according to the Polish Guide for the Care and Use of Animals (August 2, 1997).

Tissue collection and immunohistochemistry

Groups for immunohistochemistry: R + vehicle, $n = 4$; E + vehicle, $n = 4$; R + 50 μ g NPY, $n = 6$; E + 50 μ g NPY, $n = 6$. Immediately after decapitation, brains were perfused *via* both carotid arteries with 1000 ml 0.1 M phosphate buffered saline (PBS) and subsequently with 2000 ml 0.1 M PBS containing 4% paraformaldehyde (w/v) and 15% saturated picric acid solution (w/v), pH 7.4. The hypothalami and pituitaries were dissected out 20 min after the beginning of perfusion and fixed for a further 72 h by immersion in the same fixative. Hypothalami were formed in blocks of the same dimension and cutting face. Pituitaries were cut in sagittal plane at two parts. The tissue was washed with 0.01 M PBS. One hypothalamus from each group and half from each pituitary were cryoprotected in a 20% sucrose solution in 0.1 M PBS for at least two days and stored at -70°C. This material was cut on a cryostat (Jung CM 1500, Leica Instruments GmbH, Nussloch, Germany) at 30 μ m (hypothalami) and 10 μ m (pituitaries). Remaining tissues were dehydrated in graded alcohols, embedded in paraplast and then cut at 7 μ m (hypothalami) and 3 μ m (pituitaries). Hypothalami were cut in frontal plane between the septum and the mammillary body, throughout the diagonal band of Broca, the area preoptica, the organum vasculosum of the lamina terminals, the anterior hypothalamic area, the medial basal hypothalamus including the median eminence and the pituitary stalk. Hypothalamic nuclei were identified using the atlas of the sheep brain (Welento et al. 1969). Sections were processed for IHC. All sections were washed in 0.01 M and then incubated for 30 min in 0.1% hydrogen peroxide in 0.01 M PBS and 30 min in 2% preimmune lamb serum in 0.01 M PBS. Additionally cryostat sections were incubated in 0.5% Triton X-100 in 0.01 M PBS for 30 min. The hypothalami sections were incubated with primary antiserum for 6 days at 4°C, pituitary sections for 48 h at

4°C. The following antisera were used: anti-SS (ref. 19609) incubated at dilution 1:2000, anti-hGH (ref. 19558), anti-eGH (ref. 19538) incubated at dilution 1:1000 for 48 h at 4°C. All antisera were kindly donated by Dr M.-P. Dubois (I.N.R.A, Nouzilly, France). Methodological details and the specificity of these antibodies were described by Dubois (1971) and Dubois and Barry (1974). After incubation with primary antiserum the sections were rinsed in 0.01 M PBS and incubated for 2 hours at room temperature in secondary antiserum (sheep anti-rabbit IgG labelled with peroxidase, Institute Pasteur, Paris, France), dilution 1:40 with 0.1% normal lamb serum in 0.01 M PBS. Colour reaction was developed by incubating sections with 0.5% 3'-diaminobenzidine tetrachloride chromogen (Sigma, St Louis, USA) and hydrogen peroxide in 0.05 M TRIS buffer. Selected sections were additionally stained by using the silver intensification method of Liposits et al. (1984). Control staining consisted of replacing of primary antisera with the same dilution of rabbit serum. To evaluate specificity of staining for SS and GH, antisera were preadsorbed by using synthetic SS (cyclic Clin-Midy, France) or hGH and eGH (Sigma, St Louis, USA). SS antiserum was mixed with 10 µg/ml of synthetic SS, hGH or eGH antisera were mixed with 4 µg/ml hGH or eGH, preincubated for 24 h at 4°C and then used for staining instead of primary antibody. Both control staining procedures demonstrated the absence of specific staining (data not shown).

Image analysis

A projection microscope Nikon type 104 (Nikon Corporation, Yokohama, Japan) was used to analyse hypothalamic and pituitary sections. Staining was analysed using image analyse computer system "Lucia" version 3.51ab (Laboratory Imaging Ltd, Prague, Czech Rep.). Immunostained sections were projected by camera (Panasonic KR222, Matsushita Electric Industrial Co, Osaka, Japan) to a colour monitor. Pictures were adjusted for optimal contrast, fixed on the same brightness levels and saved in the buffering system. The analyses were performed under x 4 objective for the SS terminals in the ME, x 10 for SS perikarya in the periventricular nucleus (PEN) and x 40 for the somatotrophs in the adenohypophysis. Two parameters were measured: i) area fraction (percentage of total area that exhibited positive staining); ii) integral density (the sum of individual optical densities of each pixel in the area being mea-

sured, describes the amount of substance in histological sections) for each hormone in the subarea of interest using the thresholding technique. Before measurement pictures were processed by subtraction of the background and cleaning of artifacts. Frame size was kept constant for the duration of the image analysis. The analysis of SS was performed for each brain at both sides of the 3rd ventricle. The quantification of data was based on 18 measurements: 9 in every fourth mounted and stained for SS section through the PEN in the anterior hypothalamic area (0.3286 mm²/field); 9 from every second section through the medial part of the ME (delineated area). The analysis of somatotrophs was made in the 4 sections of adenohypophysis, using every 40th mounted and stained for GH section (9 fields of 0.0837 mm² measured in each section). The data from each section were averaged to obtain a mean estimate for each of the PEN, ME and adenohypophysis within each animal. The mean data were pooled to represent treatment group (E + vehicle, R + vehicle, E + NPY and R + NPY) means. These data were then analysed by non-parametric Mann-Whitney test (R vs. E) and by non-parametric Wilcoxon test (vehicle vs. NPY) test. The data are reported as the mean percentage ± SEM of the total area that exhibited positive staining and mean relative units ± SEM of integral density. Significance was defined at the $P < 0.001$ level.

Radioimmunoassay (RIA)

Groups for RIA estimations: R + vehicle, $n = 6$; E + vehicle; $n = 6$; R + 5 µg NPY, $n = 6$; E + 5 µg NPY, $n = 6$; R + 50 µg NPY, $n = 6$; E + 50 µg NPY, $n = 6$.

Growth hormone: Plasma GH was estimated in duplicate by double-antibody RIA according to Slaba et al. (1994) using pituitary derived bGH, purified by chromatography on SE-Sephadex C-50 in sodium acetate buffer for iodination and reference standards. The first antibody was produced in rabbits against pituitary-derived bGH NIDDK -GH-B-1003A. The assay detection limit was 0.034 ng corresponding to 0.68 ng/ml plasma sample. The intra-assay and inter-assay coefficients of variation (CV) averaged 5.9 and 10.2%, respectively.

IGF-I: Plasma IGF-I was determined by double-antibody RIA, according to a modified procedure described in Daughaday et al. (1980). Extraction of samples was performed using acidified ethanol and cryoprecipitation according to Justová and Hána (1993). Efficiency of extraction was approximately 80%. Anti-

body against IGF-I was prepared and kindly donated by Dr G.S.G. Spencer (Ruakura Agricultural Center, New Zealand) (Spencer et al. 1987). Recombinant of IGF-I (Calbiochem, La Jolla, USA) was used for iodination and as standard. Iodinated IGF-I was prepared by using the standard chloramine-T method. The detection limit was 0.6 ng/ml and the 50% displacement on the standard curve was 6.35 ng/ml. The intra-assay CV and the inter-assay CV were 7.3% and 10.2%, respectively.

Statistical analysis

Data are presented as mean \pm SEM for statistical analysis, significance was defined at the $P < 0.05$ level. The mean concentration of GH for individual animals was calculated from the area under the curve (the sum of trapezoid areas between the curve and the abscissa). Pulse characteristics of GH were calculated using the Pulsar Computer Program developed by Merriam and Wachter (1982) and adapted to operate on an IBM-PC. The cut-off parameters $G_{(n)}$ were settled to a 5% error rate assuming a normal distribution of data. The level of significance for differences between groups of data obtained from plasma GH analysis was calculated using the non-parametric Kruskal-Wallis test (Kruskal and Wallis 1952). The analyses of IGF-I parameters were calculated for each sheep and compared in groups by one-way analysis of variance using StatisticaTM PL computer program (StatSoft® Kraków, Poland). Comparisons between respective pairs of groups were made using the Student's *t*-test.

RESULTS

Body weight changes

The initial body weight at the age of 6 months was similar for all lambs and averaged 29.7 ± 4.6 kg. The final body weight after 17 weeks of experimental feeding (the day of slaughter) was 40.5 ± 2.5 kg for R group ($n = 10$) and 45.8 ± 5.4 kg for E group ($n = 10$). The corresponding daily body gain calculated for this period was 73 ± 23 g/day and 111 ± 27 g/day respectively.

Effect of restricted and elevated doses of protein in diet on the SS hypothalamic neuronal system, somatotrophs, GH and IGF-I in blood plasma

The localisation of irSS in hypothalamic neurones was similar in all ewes examined in both types of diet.

IrSS perikarya created a distinct centre located in the periventricular zone extending from the suprachiasmatic area in the anterior hypothalamus up to the medial basal hypothalamus, including the suprachiasmatic nucleus, PEN and paraventricular nucleus. IrSS nerve terminals in the ME were arranged in its external zone on the entire width of this organ from its anterior part to the pituitary stalk, showing greatest accumulation in the medial part. The level of protein in the diet elicited a clear-cut change in the SS perikarya and in the amount of irSS material in the ME. In R sheep, the SS perikaryonal centre displayed intense staining. Numerous cells were well filled with immunoproduct and surrounded by SS-positive axons (Fig. 1a). In this group, image analysis demonstrated that

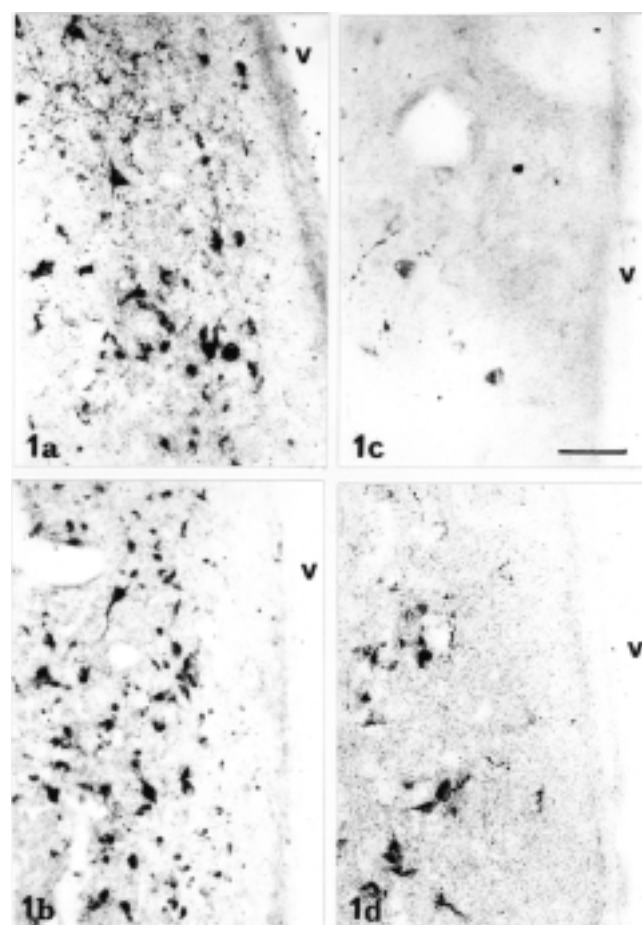


Fig. 1. Immunoreactive SS perikarya in the periventricular nucleus of two representative pairs of sheep from groups fed on R (a, b) and E (c, d) protein diets after vehicle (a, c) and NPY (50 μ g) (b, d) infusions in the 3rd ventricle of the brain. Note a high number and strong stain intensity of cells in the sheep fed on R diet (a) and the increased number and intensity of staining of cells in the sheep fed on E diet and treated with NPY (d). V-3rd ventricle, bar = 100 μ m.

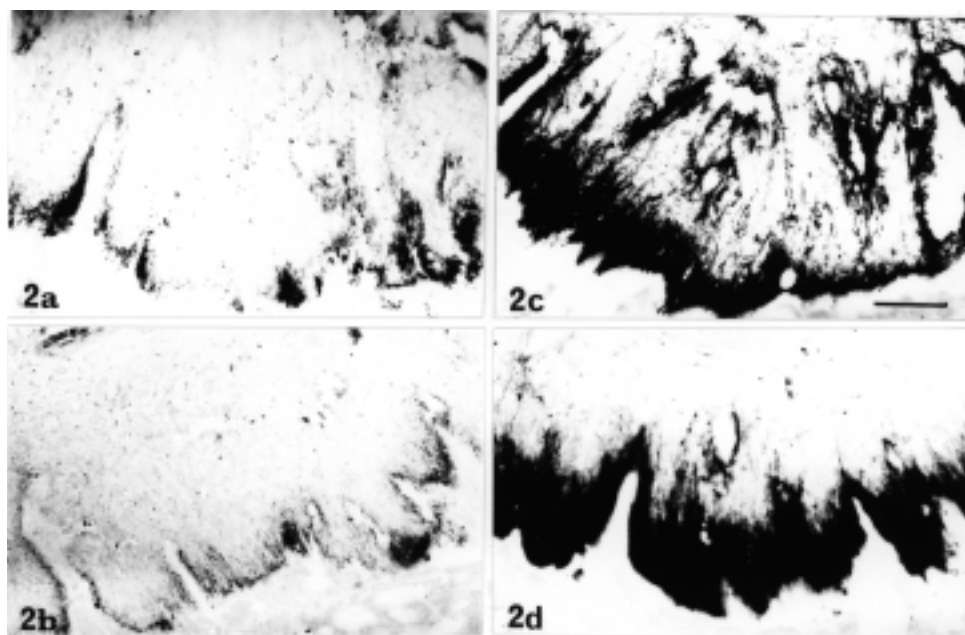
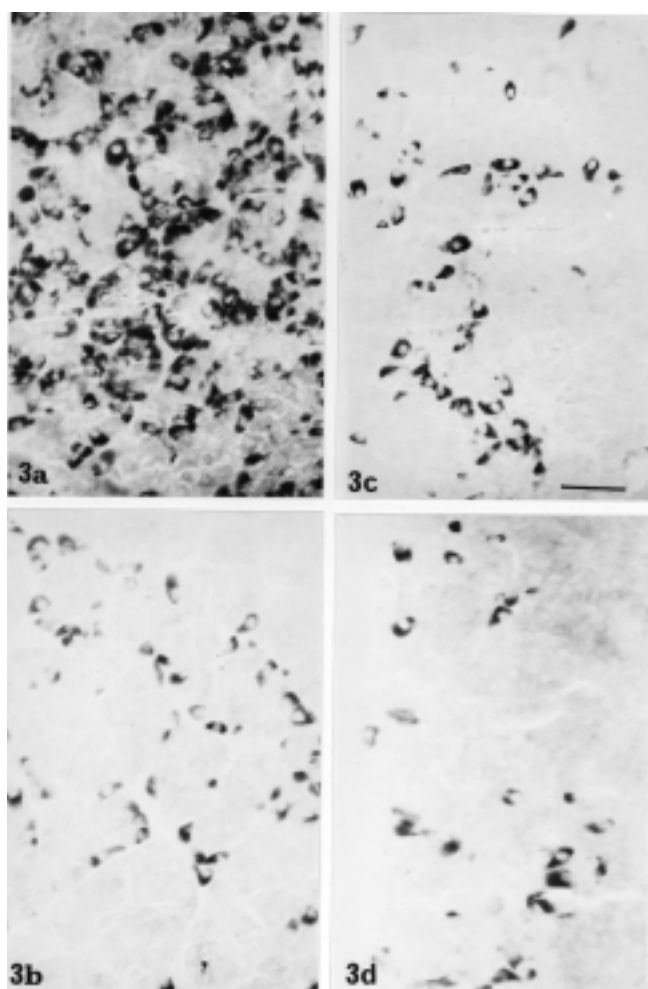


Fig. 2. Immunoreactive SS terminals in the medial part of the median eminence of two representative pairs of sheep from groups fed on R (a, b) and E (c, d) protein diets after vehicle (a, c) and NPY (50 μ g) (b, d) infusions to the 3rd ventricle of the brain. Note the low concentration of SS immunoprodukt in the sheep fed on R diet (a) and no changes after NPY infusions in sheep fed on both, E and R diets (b, d). Bar = 500 μ m.



both integral density and percentage of area containing positive staining of SS perikarya were higher ($P < 0.001$) in R than in E sheep (Fig. 4). In sheep fed E protein diet, a drastic reduction of immunostaining and visible number of SS perikarya were seen in the hypothalami (Fig. 1c). Microscopic observations of the SS located in the terminals of the ME revealed that in sheep fed R protein diet the amount of irSS material was scarce throughout all cross-sections from anterior part of the ME to the pituitary stalk (Fig. 2a). In contrast, very abundant irSS material was stored in the terminals of the ME of sheep fed E protein diet (Fig. 2c). The image analysis demonstrated that both integral density and percentage of area exhibiting positive staining for SS were significantly higher ($P < 0.001$) in E than in R sheep (Fig. 4).

The immunohistochemical analysis of somatotrophs showed that the number of cells stained positively for the presence of GH were different in pituitaries of sheep fed R or E diet (Fig. 3a, c). Both, integral density (amount of hormonal material) and percent area containing GH cells were greater ($P < 0.001$) in R than in E sheep (Fig. 4).

The long-term feeding on two different doses of protein in the diet had an effect on GH plasma levels. The

Fig. 3. Immunoreactive somatotrophs in the adenohypophyses of two representative pairs of sheep from groups fed on R (a, b) and E (c, d) diets after vehicle (a, c) and NPY (50 μ g) (b, d) infusions to the 3rd ventricle. Note the distinct diminishing of the visible cells in the sheep fed on restricted diet after NPY infusions (b). Bar = 50 μ m.

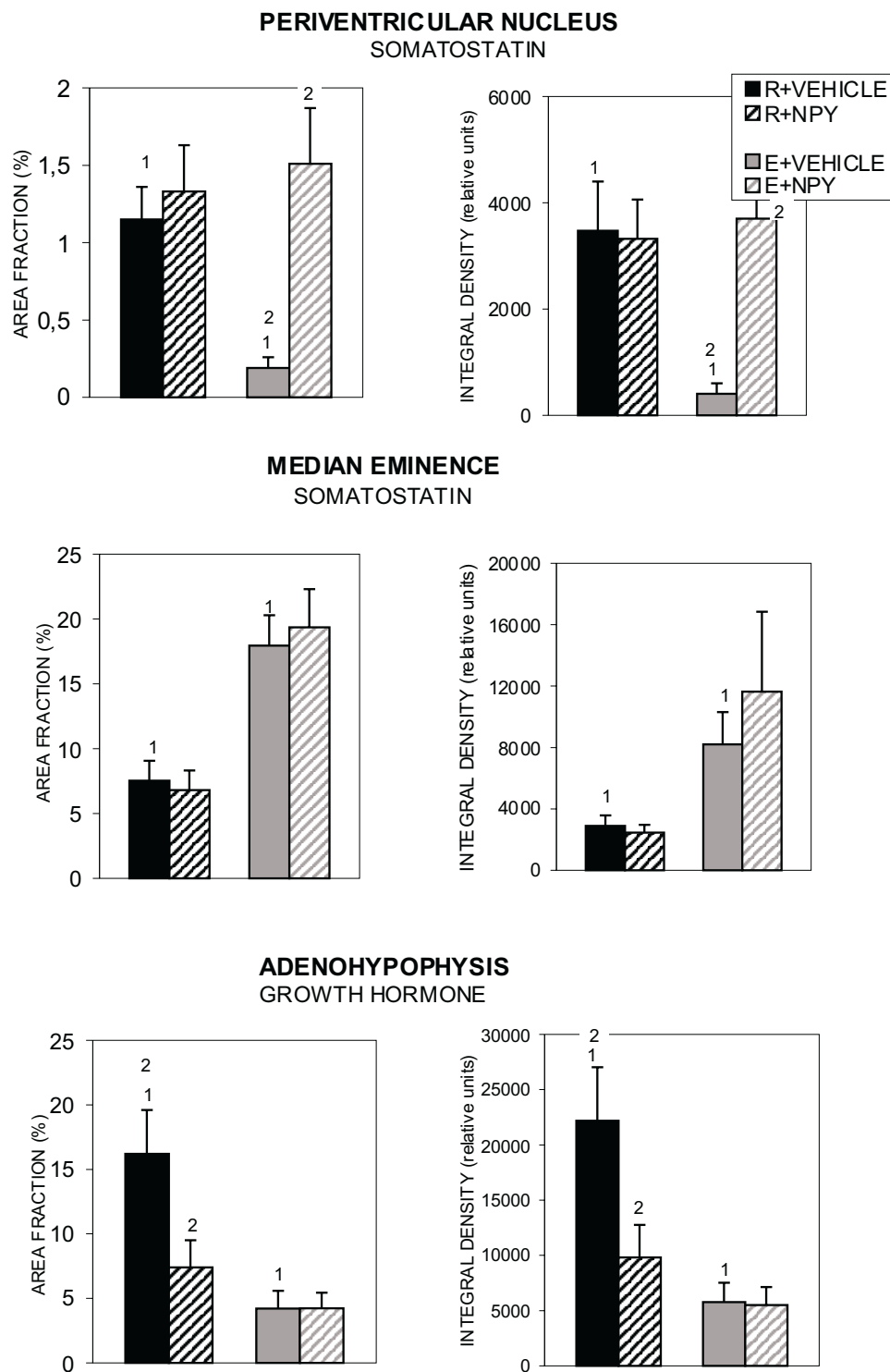


Fig.4. Percentage of total area exhibiting positive staining: area fraction (%) and integral density (relative units) for irSS in the periventricular nucleus and in the medial part of the median eminence and for irGH in the somatotrophs in the sheep from groups fed on R (black bars) and E (grey bars) diets after vehicle and NPY (50 μ g) infusions (hatched bars) to the 3rd ventricle. Means \pm SEM with different typescript (^{1,2}) differ at $P < 0.001$. The same number over bars marked the statistical significance between them.

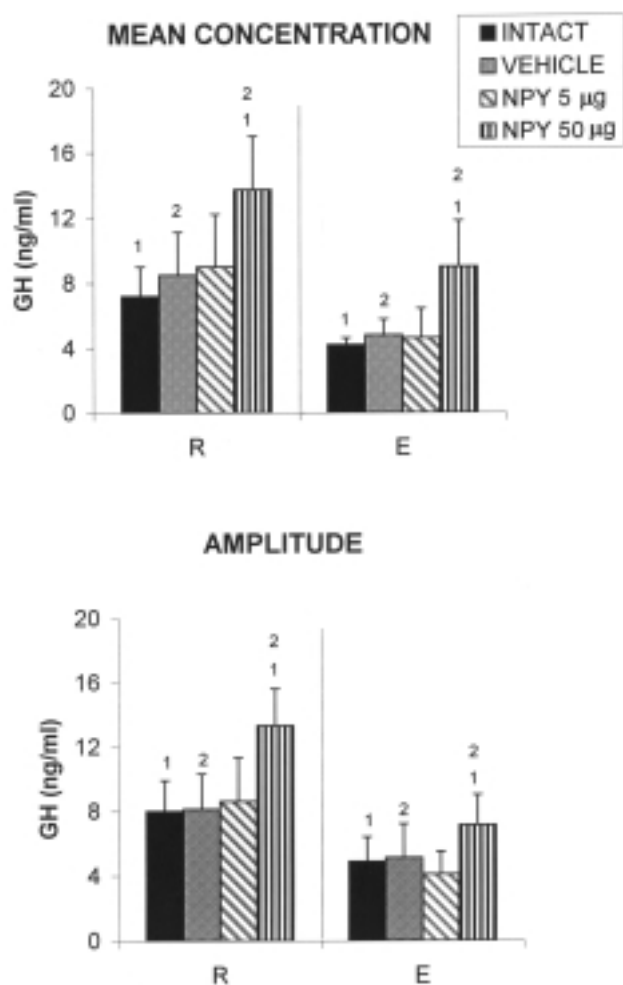


Fig. 5. Mean concentrations and mean amplitudes of GH in the blood plasma of sheep fed on R and E protein diet before application of vehicle and after vehicle infusions, after infusions of 5 µg NPY and 50 µg of NPY. Means \pm SEM with different typescripts (^{1,2}) differ at $P < 0.05$. The same number over bars marked the statistical significance between them.

mean plasma concentrations of GH were significantly higher ($P < 0.05$) in R than in E sheep (Fig. 5; 7.2 ± 1.8 vs. 4.2 ± 0.4 ng/ml). This response was associated with an increase ($P < 0.05$) in GH pulse amplitude (Fig. 5; 8.0 ± 1.9 vs. 4.9 ± 1.5 ng/ml). Neither GH pulse frequency nor mean GH interpulse intervals changed with the level of feeding (7.3 ± 1.0 vs. 7.5 ± 1.5 n/360 min and 52.4 ± 11.4 vs. 51.0 ± 11.8 min respectively for sheep R and E).

Mean concentrations of IGF-I in the blood plasma of sheep oscillated about 50 ng/ml and did not differ significantly between two nutritional groups (Fig. 6).

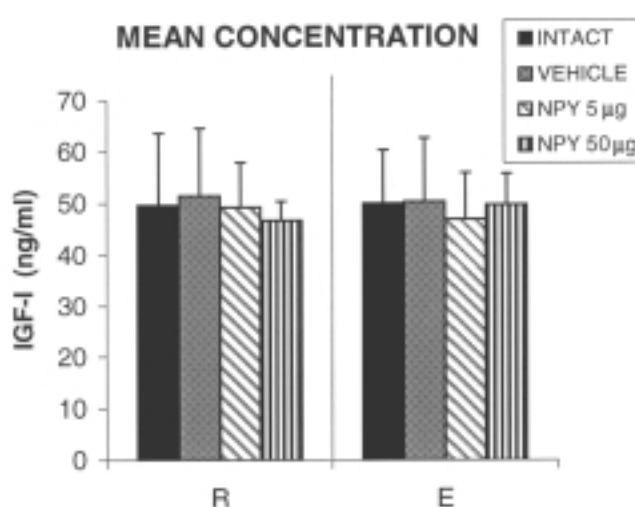


Fig. 6. Mean concentrations of IGF-I in the blood plasma of sheep fed on R and E protein diets before application of vehicle and after vehicle infusions after infusions of 5 µg NPY and 50 µg NPY to the 3rd ventricle. Means \pm SEM.

Effect of icv NPY infusions on the SS hypothalamic neuronal system, somatotrophs, GH and IGF-I in the blood plasma

The microscopic observations revealed a similar pattern of staining of the irSS perikarya (Fig. 1b) and irSS terminals in the ME (Fig. 2b) of the R + NPY sheep and R + vehicle sheep. Image analyses demonstrated similar integral density and percentage of area positively staining for SS in both groups (Fig. 4). However, marked changes were observed in the ir somatotrophs. The number of cells stained positively for the presence of GH and the intensity of the immunostaining diminished in R + NPY sheep compared to R + vehicle sheep (Fig. 3b). It was reflected by the decreased ($P < 0.001$) integral density and percentage of the area occupied by somatotrophs within the adenohypophysis of NPY-infused sheep compared to the respective control group (Fig. 4).

The intensity of the immunoreaction in the SS perikarya was enhanced in E + NPY sheep (Fig. 1d) in comparison with respective control (E + vehicle). An increase in the number of perikarya and fibers stained positively for SS presence were observed. Most of these were located in the area of the suprachiasmatic nucleus. Only fibers stained positively for SS were detected in the ventromedial nucleus. Image analysis of the periventricular zone of the suprachiasmatic area revealed higher ($P < 0.001$) integral density of SS cells and percentage of

area containing these cells (Fig. 4). Contrary to SS perikarya, the immunostaining of SS terminals in the ME and irGH in somatotrophs were similar in E + NPY and E + vehicle sheep under microscopic observations and image analysis (Figs. 2b, 3b, 4).

Intracerebroventricular administration of Ringer solution and/or a dose of 5 µg/ml of NPY did not change GH plasma concentrations and its pulse amplitude during 3 h after infusion as compared to the same values during 3 h before infusions (Fig. 5). Intracerebroventricular administration of a high dose of NPY (50 µg) resulted in a significant increase ($P < 0.05$) of the mean GH concentrations in the blood plasma and mean GH pulse amplitude measured during 3 h after infusions in both nutritional groups (Fig. 5).

Ringer and/or NPY treatment in both doses did not change mean IGF-I concentrations during 3 h after infusions as compared to the same values measured during 3 h before infusions in both nutritional groups (Fig. 6).

DISCUSSION

In the present study we have demonstrated that in sheep both the long-term nutritional manipulations and NPY infusions to the 3rd ventricle of the brain affect the irSS content in the hypothalamus, GH profiles in the pituitary gland and secretory patterns of GH in blood plasma. Previous experiments dealing with relationships between the diet and hormonal activity in sheep have largely used general restrictions of energy in feed supply. We have chosen the precisely estimated level of protein without changing the energy supply. The optimal diet recommended by Polish Nutrient Requirement for fattening Merino sheep has been established as 16.5% of crude protein and 5 MJ/kg in dry matter (Ryś 1993). Applying the diet containing 8% of protein did not change the final body weight and the daily weight gain compared with the sheep fed on the augmented dose of protein. Adaptation of ruminants to malnutrition depends on the severity and duration of the food restrictions and results from both endocrine as well as from digestive and metabolic changes (Chilliard et al. 1998). It is possible that interactions between food availability and adaptation processes in the sheep are mediated by the rumen's bacteria and protozoa which convert dietary N in microbial protein and are the main source of amino acids utilised by the host (Meyer et al. 1986).

The results of the present study show that SS neurones in the hypothalamus and GH secretion from the pituitary

gland display remarkable plasticity in response to nutritional status. This is consistent with findings from our studies on growing female Polish Lowland lambs fed on restricted protein diet from 3-rd to 9-th month of age, which showed that chronic restriction of dietary proteins augment the secretion of GH (Polkowska et al. 1996). The increased secretion of GH during restricted feeding in the sheep is likely to be attributed to the role of the SS (Thomas et al. 1991, Polkowska et al. 1996), whereas the role of growth hormone releasing hormone in these conditions seems to be of less importance (Hart et al. 1985). Microscopic observations of SS content show that long-term dietary protein restrictions intensify the immunoreaction in the cells forming SS perikarional centre and show a clear-cut reduction of SS stores in the ME. These changes can be compared with an effect of colchicine treatment which inhibits axonal transport of a peptide which is manifested by an increase in the concentration of immunoprodukt in cell soma and then unmasking of peptidergic perikarya (Sétáló et al. 1976). Thus, changes in SS content elicited by dietary restrictions can be interpreted as suppression of the SS neurones more likely related to inhibition of axonal transport of the peptide rather than to inhibition of synthesis (Polkowska et al. 1996). Limiting of protein in diets results also in the increase of the activity of somatotrophs manifested by enhancement of their number and hormonal content and in increase of GH release manifested by rise of plasma GH concentrations. The enhanced secretion of GH has been observed in adult ovariectomized sheep and lambs restricted in energy (Foster et al. 1989, Landefeld et al. 1989, Thomas et al. 1990, Thomas et al. 1991). The increase in mean GH levels due to the increase of pulse amplitude was associated with diminished SS concentrations in the hypophysial portal blood (Thomas et al. 1991) and with increased GH mRNA levels in the pituitary gland (Landefeld et al. 1989). The enhanced secretion of GH resulting from limited feeds may have maintained metabolic homeostasis by mobilising fatty acids and so conserving body proteins (Vance et al. 1992).

In contrast to other reports we have failed to observe any effect of protein restrictions in diets on plasma IGF-I concentrations. Nutritional status affects the IGF-I in ruminants by depressing of plasma concentrations (McGuire et al. 1992). However, clear differences in circulating IGF-I concentrations between restricted and temporary fed sheep were observed only under severe nutritional deficiency, i.e., starvation (Hua et al. 1993,

Hua et al. 1995, Mears 1995); negative energy balance – daily rations reduced by half for a long time (Gatford et al. 1997). Differences were not apparent when animals were only moderately underfed (Breier et al. 1986). Similarly, no differences in plasma IGF-I concentrations were reported when castrated male lambs were fed 40 days on the level of 3% or 1.7% of body weight/day (Bass et al. 1991). Changes in circulating IGF-I due to insufficient feeding are dependent not only on the daily dose but also on the kind of feeds as well as on the physiological status of the animal (age, body weight and sex) (McGuire et al. 1992, Mears 1995). It is possible that the experimental factor used in this study, the level of nutrition, age of animals and duration of experimental feeding were insufficient to affect plasma IGF-I concentrations.

The role of NPY in the mediation of nutritional signals to the somatotrophic axis in the sheep is still not known. The results of the present study show that the action of exogenous NPY on somatotrophic axis has a restraining effect on SS neurones and stimulating on GH secretion. Contrary to the findings in sheep, in rats the central administration of NPY inhibits GH secretion by elimination of its pulsatility (Catzefflis et al. 1993, Pierroz et al. 1995). It is documented that in this species NPY restrains GH by the stimulation of SS release from the nerve terminals in the ME (Rettori et al. 1990). The involvement of somatostatin in this mechanism is supported by morphological evidence demonstrated synaptic connections between NPY and SS neurones (Okada et al. 1993). It seems that in the sheep, somatostatin is also involved in the mechanism of inhibition GH secretion by NPY.

Acute NPY infusions provoked morphological changes on the level of SS synthesis (SS perikarya) and the GH release (somatotrophs) and the changes were similar to those observed after long-term nutritional manipulations. It should be stressed here that the response of the tissue altered previously by long-term treatment to the acute stimulus became visible only if the direction of changes intensifies the contrast of staining. Enhanced immunostaining of SS in perikarya could be a signal of changes in SS secretion, similar to that observed in R + vehicle treated sheep. It could be assumed that after protein restrictions and icv NPY infusions there has been arresting of SS in the cell soma visible as immunostaining. Such phenomena as decrease of intensity of immunostaining of GH - cells in R sheep along with increase of GH concentrations in blood plasma could be interpreted as rapid release of the hormone rather than diminishing

of its synthesis. Assuming that the long-term protein restrictions suppresses SS input it seems that further suppression by NPY treatment could give no contrasting staining of SS perikarya in R sheep. For the same reason NPY treatment does not alter the irSS in the ME and pituitary irGH profiles in E sheep. Taking together the above-mentioned microscopic and radioimmunological results it could be assumed that the enhanced secretion of GH resulting from NPY infusions can be due to attenuating of hypothalamic SS signal.

NPY infusions do not change the levels of IGF-I in circulating blood of sheep in either nutritional group, being measured for 3 hours after infusions. It can be supposed that lack of changes is due to restricted sensibility of IGF-I to external factors, attributed to buffering role of binding proteins. The other reason is that the monitoring of IGF-I plasma concentrations after infusion is too short. IGF-I as a relatively stable protein needs more time to respond to external stimulus. In growing heifers the response to GH injections has not been observed until ten days from the first application of the hormone (Bertozzi et al. 1998).

From this experiment we conclude that consequences of icv NPY administration on the hypothalamic SS neuronal system, GH-secreting cells and GH profiles in circulating blood are of similar character to that observed after long-term protein deprivation. Since both dietary restrictions and exogenous NPY exert a similar influence on the activation of GH secretion it could be hypothesised that NPY might be a neuromodulatory link between nutritional cues and somatotrophic axis in the sheep.

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