# *Review*

# Nutrition and hypothalamic neuropeptides in sheep: Histochemical studies

**E. Chaillou[1] and Y. Tillet[2]**

[1]"Behaviour", UMR 6175 INRA-CNRS-University of Tours-National Stud, IFR 135, INRA - Tours, Nouzilly, France and

[2]"Central Control of Ovulation", UMR 6175 INRA-CNRS- University of Tours-National Stud, IFR 135, INRA - Tours, Nouzilly, France

**Summary.** The identification and role of neuropeptides in the control of food intake and energy balance have been extensively studied in rodents, and for more than ten years, similar studies have been performed in sheep. As a photoperiodic ruminant, sheep are an interesting alternative animal model to rodents. In this review, we summarize the results obtained in sheep concerning the distribution of peptide-containing neurones in the hypothalamus and their central role in the control of food intake and energy balance, and compared them with relevant data from rodents. Even if the general organization and the role of hypothalamic neuropeptides are similar in sheep and rodents, numerous differences have been observed between these two species. In sheep, the magnocellular neurones of the paraventricular and supraoptic nuclei are characterized by the low density and the lack of galanin- and neuropeptide-Y-containing neurones, respectively. The sheep pituitary stalk presents neurones containing neuropeptides such as neuropeptide-Y or beta-endorphin, which are also found in the deep part of the infundibular nucleus. In this structure, several neuronal populations, including galanin, agouti-gene related peptide, somatostatin, are sensitive to energy balance variations, undernutrition or overfeeding, which may specifically modify neuropeptide levels in discrete neuronal subgroups. This feature is well illustrated by the number of neuropeptide-Y labelled neurones, that increases in the lateral part of the infundibular nucleus of undernourished ewes and decreases in the ventral part of overfed ewes. Conversely, after 24 hours of food deprivation, the number of neuropeptide-Y-immunolabelled neurones is unchanged in the sheep infundibular nucleus, whereas increased levels of this neuropeptide are described, in rats, by radioimmuno-assay. In conclusion, our review shows that peptide-containing neurone systems, involved in the regulation of food intake and energy balance in

sheep, are generally similar to those observed in other species, but they present specific differences according to the physiological characteristics of the animal model.

**Key words:** Ruminant, Central nervous system, Regulatory hormones

## Introduction

During the last thirty years, the central and peripheral regulations of feeding behaviour have been studied in an attempt to prevent inappropriate food intake. In the central nervous system numerous neuropeptide-containing neurones play an important role in the control of food intake and energy balance. Therefore, the regulation of food intake has been evaluated by acute or chronic central injections of peptides, agonists, and antagonists, or antiserum of the peptide. Because the nutritional status of animals is able to modify neuronal activity, the involvement of hypothalamic neuropeptides in food intake regulation has also been evaluated by measuring peptide levels, mRNA expression, or peptide-containing neurone distribution in animals submitted to different nutritional status (Smith, 1999). Most of the studies have been carried out in rodents, using wild type or selected genetic strains (for example Ob/Ob mouse, Fa/Fa rats).

Numerous neuropeptides have been studied about their potential role in the relationship between nutrition and reproduction or growth. These studies aimed to prevent obesity in humans but also to reduce the problems of reproduction and growth in farm animals. Sheep have an unusual gastric system, associated with a long period of digestion, food may be present in the rumen up to 60 hours after ingestion. This species displays seasonal variation in numerous physiological processes including reproduction, and therefore is an interesting alternative model to rodents and primates. In sheep, the use of neuroanatomical methods (immunohistochemistry or *in situ* hybridization) is a

valuable tool to further our understanding of the central nervous system that differs from that of the common model, such as rodent (Tillet, 1995). These methods allow acute identification of specific neurones among phenotypically heterogenous neuronal populations.

The immunohistochemical detection of neuropeptides is sometimes difficult because the low intra-neuronal level of these substances (mainly in rodents) necessitates treatment with colchicine. However, it appears that some peptides, like corticotropin-releasing hormone, may be detected in sheep without such a pharmacological treatment (Chaillou et al., 2002b), and modifications of nutritional status increase peptide immunoreactivity, as observed for galanin in the dorsomedial hypothalamic nucleus for example (Chaillou et al, 2003). These observations support that sheep would be an interesting model to study the distribution of neuropeptides and their role in the control of nutrition.

In this review, we assess the role, in sheep, of hypothalamic neuropeptides known to be involved in nutritional regulation in rodents (Table 1). For each neuropeptide, we describe its distribution in the hypothalamus, its central effect on food intake, and its sensitivity to nutrition. We initially focus on the peptides that stimulate food intake, and then consider food intake inhibitory peptides, and the hormonal regulatory system.

## Stimulating peptides

### Neuropeptide Y

High densities of neuropeptide Y (NPY)-immunoreactive (-ir) cell bodies and fibres are observed in the ovine hypothalamus (Antonopoulos et al., 1989a; Chaillou et al., 2002a). In colchicine-treated ewes, the highest density of NPY-ir perikarya is found in the infundibular nucleus, in which two subpopulations are distinguished; some positive immunoreactive neurones are present in the median eminence and in the pituitary stalk (Fig. 1, Chaillou et al., 2002a), whereas NPY-ir neurones seem to be homogenously distributed in the

**Table 1.** Summary of the studies that describe the hypothalamic distribution of the neuropeptides, their central effects on food intake and their sensitivity to nutrition in sheep.

| PEPTIDE | DISTRIBUTION | FOOD INTAKE | NUTRITIONAL SENSITIVITY |
|---|---|---|---|
| AGRP | Henry et al., 2001a; Adam et al., 2002; Sorensen et al., 2002 | ? (in rat, +) | Henry et al., 2001a; Adam et al., 2002 |
| Alpha-MSH | No published data | ? (in rat, -) | No published data |
| Beta-End | Antonopoulos et al., 1989b; Whisnant et al., 1992 | ? (in rat, +) | Baile et al., 1987; Prasad et al., 1993 |
| CART | Henry et al., 2001a; Adam et al., 2002 | ? (in rat, -) | Henry et al., 2001a; Adam et al., 2002 |
| CCK | Antonopoulos et al., 1987; Marson et al., 1987 | -, Della Fera and Baile, 1979 | Scallet et al., 1985, Holmberg and Malven, 1997 |
| CGRP | Herbison et al., 1993 | 0/+, Bueno et al., 1986 (in rat, -) | No published data |
| CRH | Paull et al., 1982; Kolodziejczyk et al., 1983; Palkovits et al., 1983 | -, Ruckebush and Malbert, 1986; Sunagawa et al., 2000; Weisinger et al., 2000 | Chaillou et al., 2000, 2002b |
| Dynorphin/ Enkephalin | Marson et al., 1987; Matthews et al., 1992 | +, Baile et al., 1987 | Scallet et al., 1985; Henry et al., 2000; Iqbal et al., 2003 |
| Galanin | Chaillou et al., 1998, 1999 | ? (in rat, +) | Barker-Gibb and Clarke, 1996; Chaillou et al., 2003 |
| MCH | Tillet et al., 1996 | +, Whitlock et al., 2005 | Henry et al., 2000; Chaillou et al., 2003 |
| Neurotensin | Antonopoulos et al., 1989b | ? (in rat, -) | No published data |
| NPY | Antonopoulos et al., 1989a; Chaillou et al., 2002a | +, Miner et al., 1989; Sunagawa et al., 2001 | Ober and Malven, 1992; McShane et al., 1993; Barker-Gibb and Clarke, 1996; Adam et al., 1997; Polkowska and Gladysz, 2001; Chaillou et al., 2002a |
| Orexin | Archer et al., 2002; Iqbal et al., 2001a | + Sartin et al., 2001 | Archer et al., 2002; Iqbal et al., 2003 |
| SRIF | Papadopoulos et al., 1986; Bruneau and Tillet, 1998; Willoughby et al., 1995 | -, Spencer and Fadlalla, 1989 | Thomas et al., 1991; Henry et al., 2001b |
| TRH | McDonald et al., 1993 | -, Ruckebush and Malbert, 1986 | No published data |
| Urocortin | Cepoi et al., 1999 | -, Sunagawa et al., 2000; Weisinger et al., 2000; Holmberg et al., 2001 | No published data |

When the effects on food intake have not been described in sheep or differ from those found in rat, they are specified: ?, no data; -, inhibition; +, stimulation, 0, no effect. (adapted from Smith, 1999).

arcuate nucleus of rodents (Chronwall et al., 1985; Nakagawa et al., 1985). NPY-ir cell bodies are also found in the bed nucleus of the stria terminalis, the medial preoptic area, the anterior hypothalamic area, the rostral part of the paraventricular nucleus, the dorsomedian nucleus, and the tuberomammillary nucleus (Chaillou et al., 2002a). In adult ovariectomized (ovx) ewes, NPY-ir fibres are localised in numerous hypothalamic structures, the highest densities being seen in the paraventricular and infundibular nuclei, and in the median eminence (Chaillou et al., 2002a).

An intracerebroventricular (icv) injection of NPY stimulates cumulative food intake in satiated growing prepuberal sheep (Miner et al., 1989) and ovx ewes fed on alfalfa chaff (Sunagawa et al., 2001), and blocks the effects of satiety factors, like ruminal distension or intraruminal infusion of propionate, in prepuberal sheep (Miner et al., 1990).

The number of NPY-ir neurones in the infundibular nucleus is similar between *ad libitum* fed and 24-hour food-deprived adult ovx ewes (Chaillou et al., 2002a). In rats, the stimulatory effect of deprivation was described by radioimmuno-assay (RIA) on push-pull samples (Kalra et al., 1991), or on micro-dissected samples (Sahu et al., 1988; Beck et al., 1990).

In sheep, there have been numerous studies concerning the sensitivity of the NPY hypothalamic system to nutrition. All these studies showed an increase of NPY after food restriction or undernutrition. In growth retarded ovx sheep, Ober and Malven (1992) found an increase of NPY level throughout the hypothalamus, measured by RIA on micro-dissected samples. In a study using densitometry on immuno-labelled sections, Polkowska and Gladysz (2001) observed more NPY-ir in the periventricular nucleus of lambs fed restricted protein concentration than in those fed elevated protein concentration. Furthermore, Barker-Gibb and Clarke (1996) observed an increase of NPY-ir in the median eminence-arcuate nucleus, preoptic area and paraventricular nucleus of adult undernourished ovx ewes. The number of NPY-ir cell bodies is enhanced by undernutrition in the infundibular nucleus of adult ovx ewes (Barker-Gibb and Clarke, 1996), this effect being specific to the lateral part of the nucleus (Fig. 2, Chaillou et al., 2002a). The effect of nutrition on the NPY neuronal system was also evaluated by measuring mRNA expression after *in situ* hybridization. An enhancement of NPY mRNA was found in the infundibular nucleus of undernourished ovx lambs (McShane et al., 1993) and undernourished castrated male sheep with or without protein supplement (Adam et al., 1997). Moreover, the level of NPY mRNA increased in the arcuate nucleus of adult male castrated sheep after 4-days of fasting (i.e acute negative energy balance), compared to *ad libitum* fed animals (Adam et al., 2002).

*Ad libitum* refeeding of adult ovx ewes that had previously been undernourished or fed at maintenance rates, led to a decreased number of NPY-ir neurones in the infundibular nucleus, specifically in its ventral part (Fig. 2, Chaillou et al., 2002a).

## Opioids

### Beta-endorphin and proopiomelanocortin

In the ovine hypothalamus, beta-endorphin (beta-End)-containing perikarya are located in the infundibular nucleus (Fig. 3, Antonopoulos et al., 1989b), the periventricular nucleus and around the mammillary recess (Whisnant et al., 1992). Beta-End-ir fibres are present in the same areas as the perikarya and in the ventromedial nucleus (Whisnant et al., 1992).
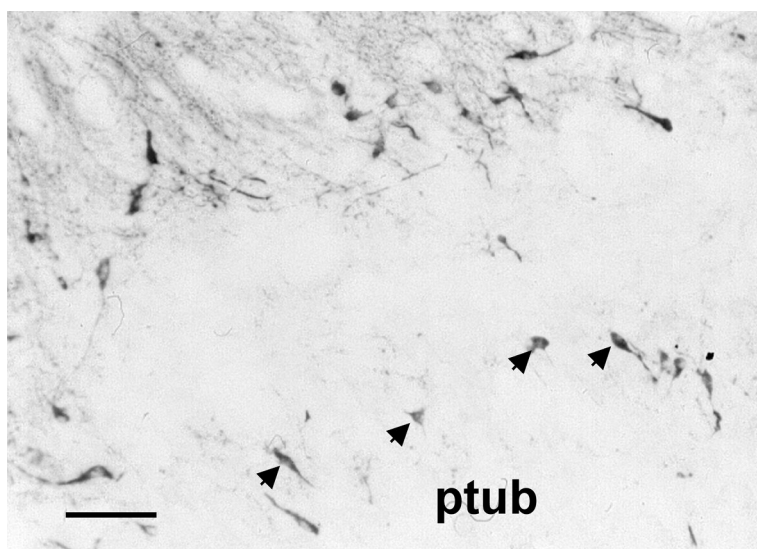


ptub

**Fig. 1.** Distribution of the NPY-immunoreactive neurones in the profound infundibular nucleus. Note the presence of labelled neurones (arrows) in the median eminence and pituitary stalk (ptub, pars tuberalis). Scale bar: 100 µm.

In rats, beta-End produces a dose- and time-dependent increase in food intake after ventricular injection (Silva et al., 2001). In sheep, the role of beta-end in the regulation of food intake is not known, but the impact of nutrition on the peptide or its precursor, proopiomelanocortin (POMC), has been studied.

The concentrations of beta-End measured in micro-punched dorsomedial hypothalamus and posterior hypothalamus of fed sheep are lower than fasted (4-hours) sheep (Baile et al., 1987). In feed-restricted lambs (208 days at 20% of National Research Council Requirements), the concentration of beta-End secreted by the posterior-lateral median eminence is lower than in fully fed controls (Prasad et al., 1993).

In sheep, the POMC mRNA are located in the arcuate nucleus (McShane et al., 1993), where the peptides, beta-End and alpha-MSH, are also found (Fig. 3). The expression of POMC (mRNA density evaluated on *in situ* hybridized sections) is lower in the arcuate nucleus of fed restricted ewe lambs than in those of fed control (McShane et al., 1993). This down-regulation of POMC mRNA expression, by feeding restriction, is neither observed in adult male castrated sheep after 4-days of fasting (Adam et al., 2002), nor in adult thin ovx ewes (animals fed about 400g of lucerne hay for 8 months, Henry et al., 2000) compared with their respective controls.

### Enkephalin and Dynorphin

Enkephalin-ir cell bodies are present in the parvocellular part of the paraventricular nucleus, in the dorsomedial, ventromedial, lateral and anterior hypothalamic areas (Marson et al., 1987). With *in situ* hybridization, the highest density of preproenkephalin mRNA is found in the paraventricular and suprachiasmatic nuclei of the hypothalamus (Matthews et al., 1992). In the hypothalamus of ovx ewes, prodynorphin mRNA-containing cells are mainly observed in the supraoptic nucleus and the retrochiasmatic subdivisions of the supraoptic, paraventricular, periventricular and ventromedial nuclei. Low numbers of labelled cells are seen in the preoptic, anterior, dorsomedial and posterior hypothalamic areas, and the zona incerta (Iqbal et al., 2003). Dynorphin-A is found in perikarya located in the supraoptic nucleus (Marson et al., 1987).

These two neuropeptides, and their analogues, stimulate food intake after an icv injection in satiated sheep (Baile et al., 1987).

The level of met-enkephalin radioimmuno-assayed in the mediobasal hypothalamus is higher in 4- or 24-hours fasted adult male sheep than in fed controls (Scallet et al., 1985). It is important to distinguish the different neuropeptide-containing areas, since the nutritional sensitivity of enkephalin depends on the hypothalamic nucleus. In thin ovx ewes, the number of enkephalin mRNA-containing cell bodies is lower in the periventricular nucleus, whereas it is higher in the paraventricular nucleus and the ventromedial hypothalamic area than in those of fat ovx control (Henry et al., 2000). The expression of prodynorphin mRNA is not affected by food restriction or food supplementation in the periventricular, paraventricular and ventromedial hypothalamic nuclei of ovx ewes
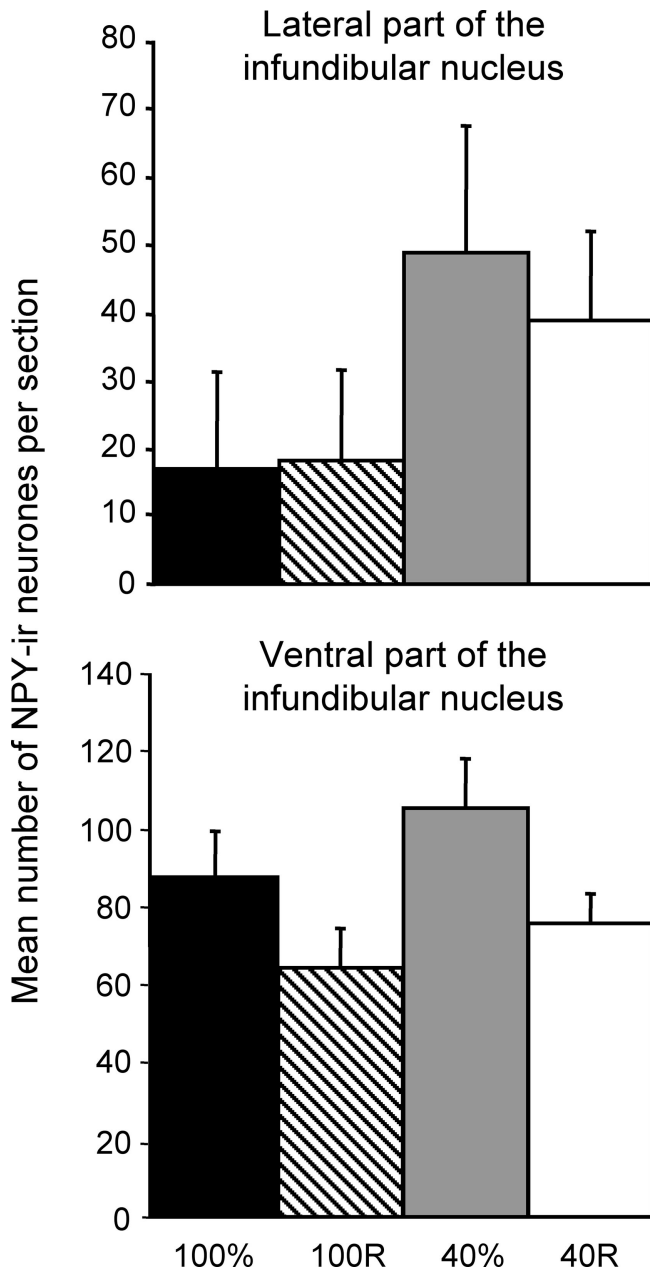


**Fig. 2.** Effect of undernutrition (40% of the nutritional requirements) and *ad libitum* refeeding on the number of NPY-immunoreactive neurones counted in the lateral (top) and ventral (bottom) parts of the infundibular nucleus. Note that undernutrition and *ad libitum* refeeding act specifically on the lateral (p=0.0001) and ventral (p=0.02) parts of the infundibular nucleus, respectively (adapted from Chaillou et al., 2002a).

(Iqbal et al., 2003).

*Galanin*

In adult ovx ewes, galanin-ir perikarya are localised in the medial preoptic area and in the caudal part of the infundibular nucleus, galanin-ir fibres are observed in the external layer of the median eminence, the mediobasal hypothalamus, and the periphery of the supraoptic and paraventricular nuclei (Chaillou et al.,

1999). In adult ewes treated with colchicine, galanin-ir cell bodies are present in higher densities in the medial preoptic area and in the rostral and caudal parts of the infundibular nucleus. The presence of these subpopulations is confirmed by the co-localization of galanin and tyrosine hydroxylase only in neurones of the caudal part of the infundibular nucleus (Chaillou et al., 1998). After colchicine treatment, labelled neurones are also seen in the supraoptic and paraventricular nuclei and in the dorsomedial hypothalamus (Chaillou et al.,
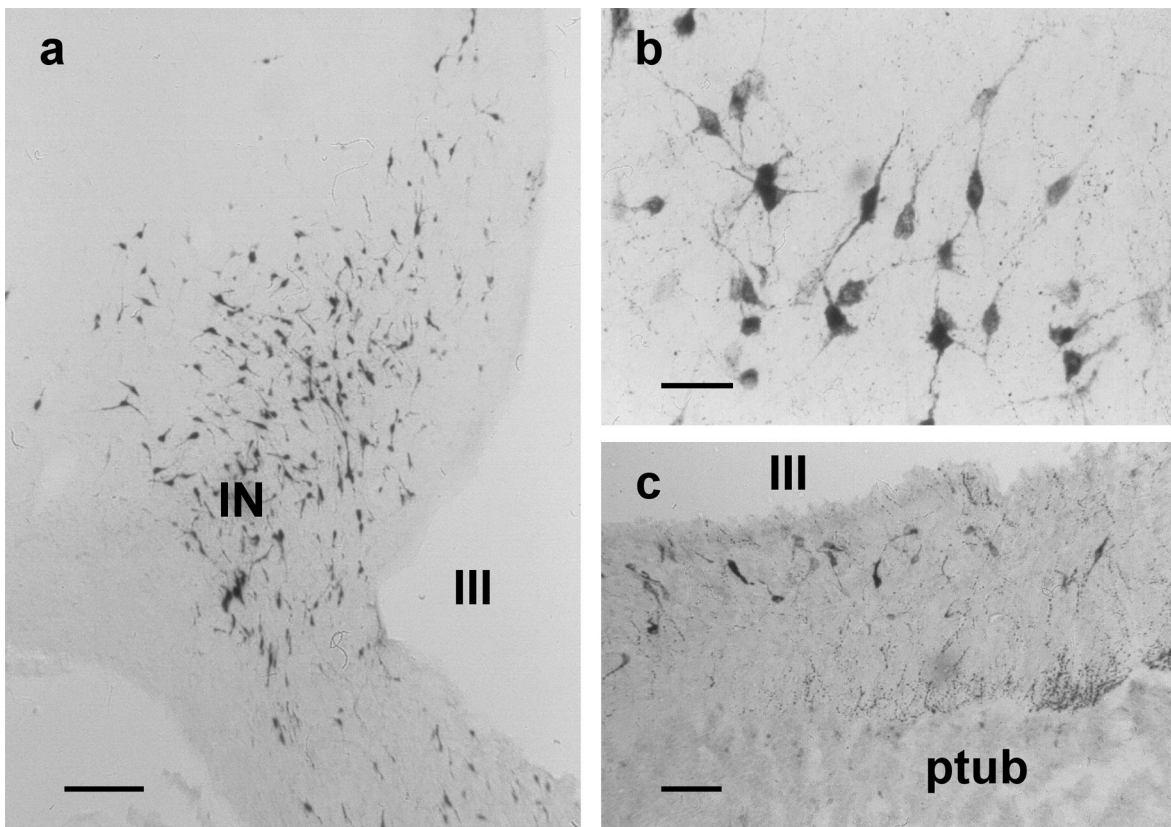


**Fig. 3.** Beta-End-immunoreactive neurones observed in the infundibular nucleus of colchicine-treated ewe **(a, b)**. The labelled neurones are distributed throughout the nucleus, and especially in its medio-ventral part **(c)**, like its precursor POMC peptide. (III, third ventricle; IN, infundibular nucleus; ptub, pars tuberalis). Scale bar: a, 200 µm; b, 50 µm; c, 100 µm.
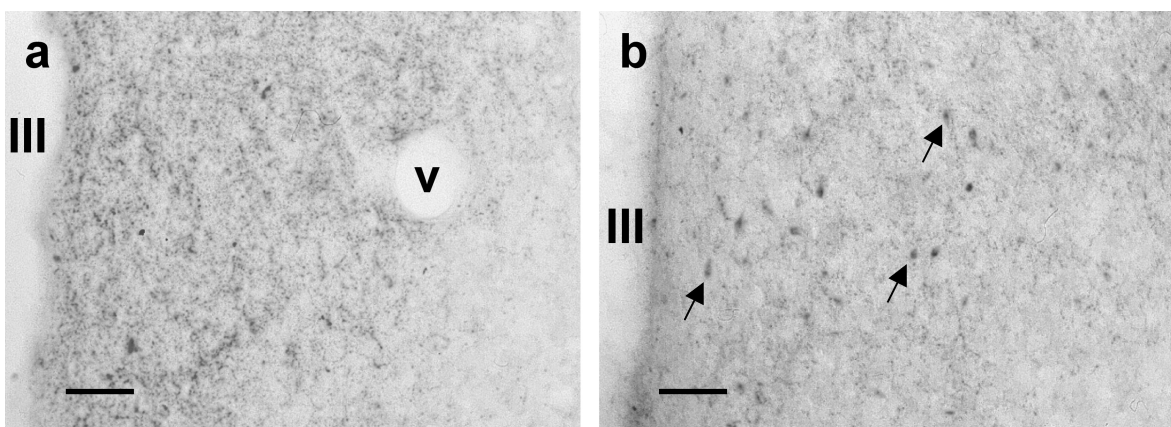
**Fig. 4.** Galanin-immunoreactive neurones observed in the dorsomedial hypothalamic nucleus of *ad libitum* refed OVX ewes after a long period of **(a)** feeding at maintenance rate or **(b)** undernutrition. Note the difference of density of neurones (arrows) between the two diets. (III, third ventricle; v, blood vessel). Scale bar: 100 µm.

1999).

The implication of galanin in the regulation of feeding behaviour has not been demonstrated in sheep. In rodents, this neuropeptide has a stimulating effect on food intake (Tempel et al., 1988; Edwards et al., 1999). However, the fasting effect was studied in adult ovx ewes, 24-hours of food deprivation has no effect on the number of galanin-ir cells compared to *ad libitum* fed controls, neither in the preoptic area nor the infundibular nucleus (Chaillou et al., 2003).

The involvement of this hypothalamic neuropeptide has been partially demonstrated in studies that evaluate the effect of undernutrition on the peptide level. Galanin-ir, quantified by optic density, was greater in the mediobasal hypothalamus of adult ovx ewes that were undernourished for 17 months than in *ad libitum* fed controls, but the number of galanin-immunostained cells was unchanged (Barker-Gibb and Clarke, 1996). In adult ovx ewes, long-term undernutrition leads to an increased number of galanin-ir cells in the infundibular nucleus and the dorsal hypothalamic area (Chaillou et al., 2003). The same effect is also found in *ad libitum* refed adult ovx ewes, after a long period of undernutrition or feeding at maintenance rate, in the preoptic area, the infundibular nucleus and the dorsal hypothalamic area (Fig. 4, Chaillou et al, 2003).

### Melanin-concentrating hormone

In the sheep hypothalamus, melanin-concentrating hormone (MCH)-ir perikarya (Tillet et al., 1996) as well as MCH mRNA-containing neurones (Henry et al., 2000) are found in the lateral hypothalamic area, around the fornix and under the mammillothalamic tract.

An icv injection of MCH leads to an increase in plasma osmolality and plasma glucose in ovariectomized ewes (Parkes, 1996) and enhances cumulative feed intake in catrasted male sheep (Whitlock et al., 2005).

The observed effects of undernutrition on MCH-containing neurones depend upon the histochemistry methods (Fig. 5). In ovx adult thin undernourished ewes, only the number of silver grains per cell, not the number of MCH mRNA-containing cells, is increased in the lateral hypothalamic area, compared to *ad libitum* fed fat ewes (Henry et al., 2000). However, the number of MCH-ir neurones is not affected by long-term undernutrition in adult ovx ewes (Chaillou et al., 2003). Moreover, the number of MCH-ir neurones is not modified by over-feeding or by 24-hours of food deprivation, in the same animal model (Chaillou et al., 2003).

### Agouti-gene related peptide

Agouti-gene related peptide (AGRP) mRNA is localized in the arcuate nucleus of adult ovx (Henry et al., 2001a), intact ewes (Sorensen et al., 2002), and adult male castrated sheep (Adam et al., 2002).

This neuropeptide is known to stimulate food intake in rodents (Table 1) but no data are available in ovine species.

In the arcuate nucleus, the expression of AGRP mRNA is up-regulated in thin adult ovx ewes compared to *ad libitum* fed animals, in which the expression is not detectable (Henry et al., 2001a). In adult male castrated sheep, the effects of acute negative energy balance were studied after 4-days of fasting. In this case, the expression of AGRP mRNA was increased (Adam et al., 2002).

### Orexin

Orexin positive-ir cells are observed in the lateral hypothalamic area, perifornical nucleus and zona incerta. The highest density is seen in the dorsomedial
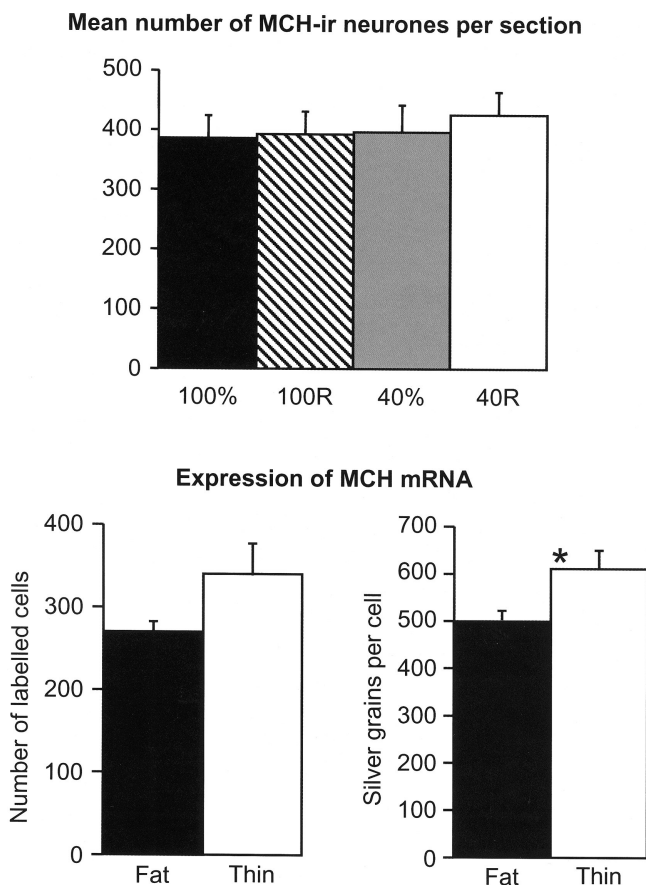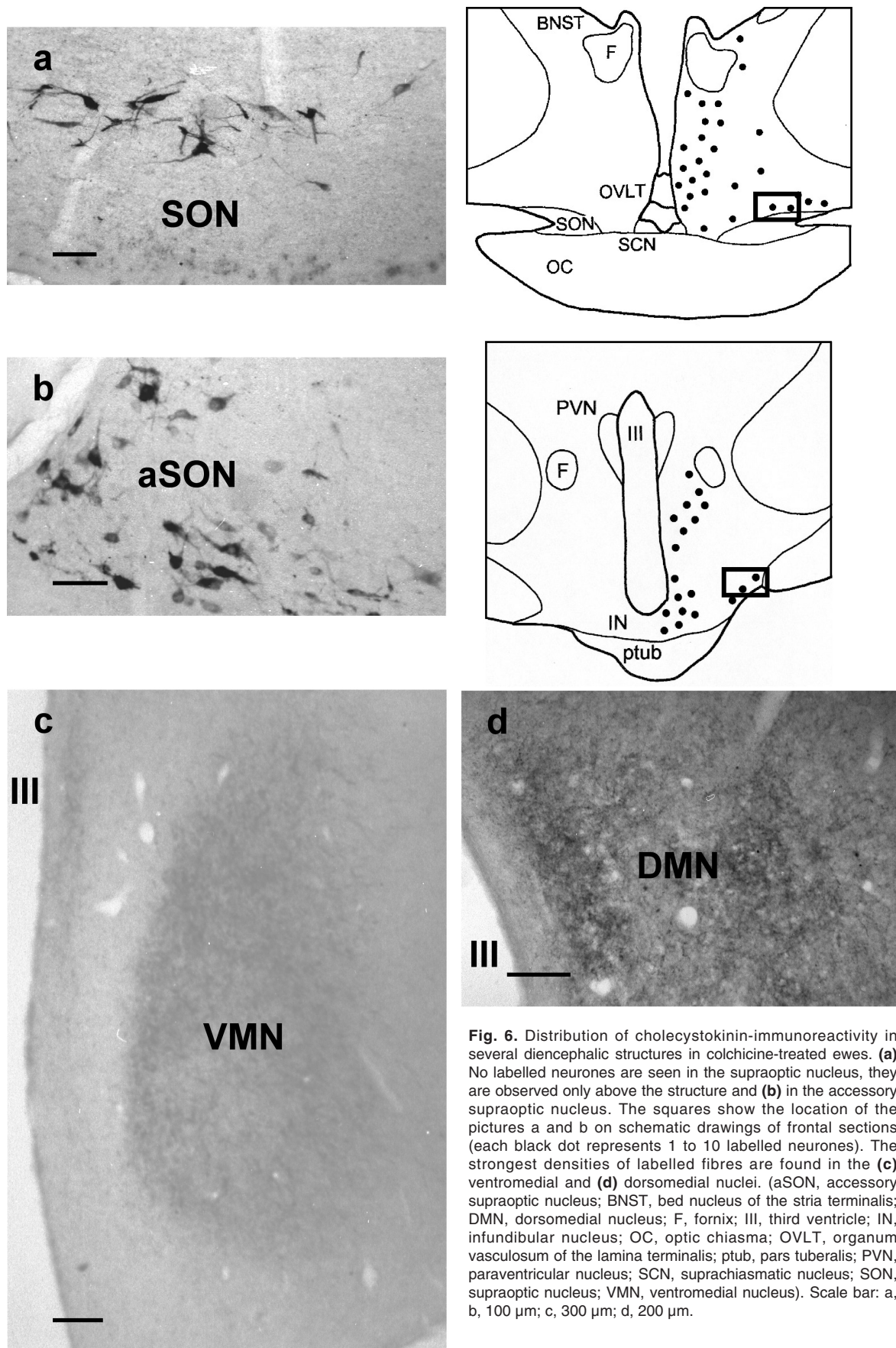
**Mean number of MCH-ir neurones per section**



**Expression of MCH mRNA**



**Fig. 5.** Nutritional effects on the number of MCH-immunoreactive neurones and MCH mRNA expression, in the lateral hypothalamic area. Note that the number of MCH-ir neurones is unchanged, either by undernutrition (comparison of 100% and 100R, with 40% and 40R), or *ad libitum* refeeding (comparison of 100% and 40%, with 100R and 40R) (adapted from Chaillou et al., 2003). The sensitivity of MCH neuronal system to nutrition is only observed when mRNA expression is evaluated by the number of silver grains per cell. This latter increases in thin animals (*, p<0.05) adapted from Henry et al. (2000).

**Fig. 6.** Distribution of cholecystokinin-immunoreactivity in several diencephalic structures in colchicine-treated ewes. **(a)** No labelled neurones are seen in the supraoptic nucleus, they are observed only above the structure and **(b)** in the accessory supraoptic nucleus. The squares show the location of the pictures a and b on schematic drawings of frontal sections (each black dot represents 1 to 10 labelled neurones). The strongest densities of labelled fibres are found in the **(c)** ventromedial and **(d)** dorsomedial nuclei. (aSON, accessory supraoptic nucleus; BNST, bed nucleus of the stria terminalis; DMN, dorsomedial nucleus; F, fornix; III, third ventricle; IN, infundibular nucleus; OC, optic chiasma; OVLT, organum vasculosum of the lamina terminalis; ptub, pars tuberalis; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus; VMN, ventromedial nucleus). Scale bar: a, b, 100 µm; c, 300 µm; d, 200 µm.

hypothalamic nucleus, whereas a few scattered cells are found in the anterior hypothalamic area (Iqbal et al., 2001a). Preproorexin mRNA, localised by *in situ* hybridization, is observed in the lateral hypothalamic area, zona incerta (Archer et al., 2002), dorsomedial hypothalamic, perifornical and posterior hypothalamic areas (Iqbal et al., 2003). An icv injection of orexin-B increases food intake in castrated male sheep (Sartin et al., 2001), but the expression of preproorexin is not influenced by nutrition in adult Soay castrated male sheep (Archer et al., 2002) or ovx ewes (Iqbal et al., 2003).

**Inhibitory peptides**

*Cholecystokinin*

In sheep diencephalon, cholecystokinine (CCK)-ir neurones are observed in the medial preoptic and anterior hypothalamic areas, periventricular and suprachiasmatic nuclei, and dorsocaudal hypothalamic area (Antonopoulos et al., 1987; Marson et al., 1987). Contrary to rodents (Vanderhaegen, 1985), no labelled cell bodies are seen in the hypothalamic magnocellular nuclei in colchicine treated ewes (Fig. 6a), except in the accessory supraoptic nucleus (Fig. 6b). In adult ovx ewes, the highest densities of CCK-ir fibres are observed in the medial preoptic area, anterior hypothalamic area, and in the ventromedial (Fig. 6c), dorsomedial (Fig. 6d) and dorsocaudal nuclei of the hypothalamus. Rare labelled fibres are present in the infundibular nucleus of adult ovx ewes, whereas this structure contains very high densities of labelled fibres in rats (Vanderhaegen, 1985).

Icv perfusion of CCK for 3 hours in 2-, 4-, 8- or 24-hours fasted sheep inhibits food intake, without affecting water intake (Della-Fera and Baile, 1979). The role of this peptide was confirmed by icv injection of CCK antibody, which stimulates food intake in satiated castrated male sheep (Della-Fera and Baile, 1981).

In the anterior hypothalamic area, the level of CCK/gastrin-like immunoreactivity, measured by RIA, is higher in satiated sheep than in 2-, 4-, or 24-hours fasted sheep (Scallet et al., 1985). In the hypothalamus of growth-retarded castrated male lambs, the expression of CCK mRNA, evaluated on northern blot samples, is unchanged compared to non-growth-retarded animals (Holmberg and Malven, 1997).

*Corticotropin-releasing hormone and urocortin*

In the ovine hypothalamus, corticotropin-releasing hormone (CRH)-ir neurones are only observed in the paraventricular nucleus (Paull et al., 1982). Labelled fibres are mostly observed in the external median eminence (Paull et al., 1982, Kolodziejczyk et al., 1983) where the highest density of CRH-ir in micro-punched samples of hypothalamic regions is found (Palkovits et al., 1983). An icv injection of CRH leads to decreased food and water intake in hay fed sheep (Ruckebush and Malbert, 1986), and in ovx ewes after a continuous infusion for 4 (Weisinger et al., 2000) and 5 days (Sunagawa et al., 2000). In castrated male sheep, the expression of CRH mRNA in the paraventricular nucleus, is not modified during insulin-induced hypoglycemia (Adam and Findlay, 1998), while the concentration of CRH in the hypophysial-portal circulation is enhanced in similar sheep models (Engler et al., 1989). In adult ovx ewes, the number of CRH-ir neurones in the paraventricular nucleus is the same between *ad libitum* fed and 24-hours food-deprived animals (Chaillou et al., 2000). Conversely, in this same structure, a long-term undernutrition period results in an increased number of CRH-ir neurones (Fig. 7), without modifying plasma cortisol concentration (Chaillou et al., 2002b).

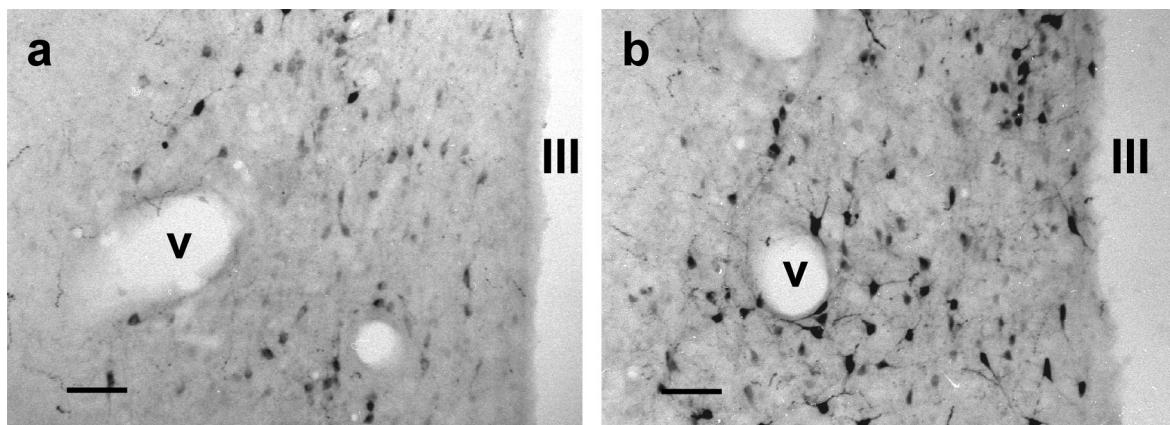The distribution of urocortin mRNA was described



**Fig. 7.** CRH-immunoreactive neurones located in the paraventricular nucleus of *ad libitum* refed OVX ewes after a long period of **(a)** feeding at maintenance rate or **(b)** undernutrition. Note the difference of density of neurones between the two diets. (v, blood vessel; III, third ventricle). Scale bar: 100 μm.

by *in situ* hybridization in sheep, with the strongest signal found in the Edinger-Westphal nucleus extending to the posterior hypothalamic area (Cepoi et al., 1999).

In ovx ewes, food intake is decreased by icv urocortin continuously infused for 4 days (Sunagawa et al., 2000; Weisinger et al., 2000) or infused in a linearly increasing manner (Holmberg et al., 2001). There are no data concerning nutritional sensitivity of the urocortin neuronal system in sheep.



**Fig. 8.** Nutritional sensitivity of the CART neuronal system observed on the mRNA expression. Note that the effects have been observed according to the measure (number of labelled cells or the number of silver grains per cell). Adapted from Adam et al. (2002) and Henry et al. (2001a).

### Somatostatin

In the hypothalamus of adult ewes treated with colchicine, somatostatin (SRIF)-ir perikarya are observed in the anterior hypothalamic area, in the suprachiasmatic, supraoptic, paraventricular, infundibular, and around the ventromedial nuclei (Papadopoulos et al., 1986). The mRNA coding for the prepro-SRIF are observed in the same nuclei of adult ovx ewes (Bruneau and Tillet, 1998). SRIF-ir fibres are found in the dorsal ventromedial nucleus, arcuate nucleus and the median eminence (Willoughby et al., 1995).

An icv injection of anti-SRIF serum increases food intake in sheep, suggesting an inhibitory effect of SRIF on feeding behaviour (Spencer and Fadlalla, 1989).

In sheep fed a restricted diet for 20 weeks, the hypophysial portal concentration of SRIF is lower than in fed control sheep (Thomas et al., 1991). This decrease seems to be associated with decreased SRIF expression, since in ovx ewes fed a restricted diet for 7 months, the level of SRIF mRNA, measured by *in situ* hybridization, is lower in the rostral periventricular and the ventromedial hypothalamic nuclei, compared to that measured in *ad libitum* fed controls (Henry et al., 2001b).

### Cocaine- and amphetamine-regulated transcript

The expression of cocaine- and amphetamine-regulated transcript (CART) mRNA is abundant throughout the ovine hypothalamus. CART mRNA-containing cells are observed in the retrochiasmatic, periventricular, paraventricular, and dorsomedial nuclei, and in the lateral and posterior hypothalamic areas of adult ovx ewes (Henry et al., 2001a). In adult castrated male sheep, expression of CART mRNA is found in the arcuate, paraventricular, and ventromedial hypothalamic nuclei, and in the median eminence (Adam et al., 2002).

Whereas the inhibitory effect of CART found in rodents after an icv injection (Table 1) has not been described in sheep, the effect of nutrition on its mRNA expression has been studied.

In adult castrated male sheep, 4-days of fasting tends to down-regulate the expression of CART mRNA in the arcuate nucleus, but has no effect on the amount of CART mRNA in the paraventricular nucleus (Adam et al., 2002). These data differ from those in adult ovx ewes, in which food restriction leads to a decreased number of CART mRNA-containing cells in the retrochiasmatic, paraventricular and dorsomedial nuclei, and in the lateral hypothalamic area, whereas CART mRNA is not detected in the arcuate nucleus (Henry et al., 2001a). Concomitantly, the number of CART silver grains per cell decreases in the retrochiasmatic and paraventricular nuclei, and increases in the posterior hypothalamic area after food restriction (Henry et al., 2001a) (Fig. 8). The inconsistent results reported by Henry et al. (2001a) and Adam et al. (2002) could be
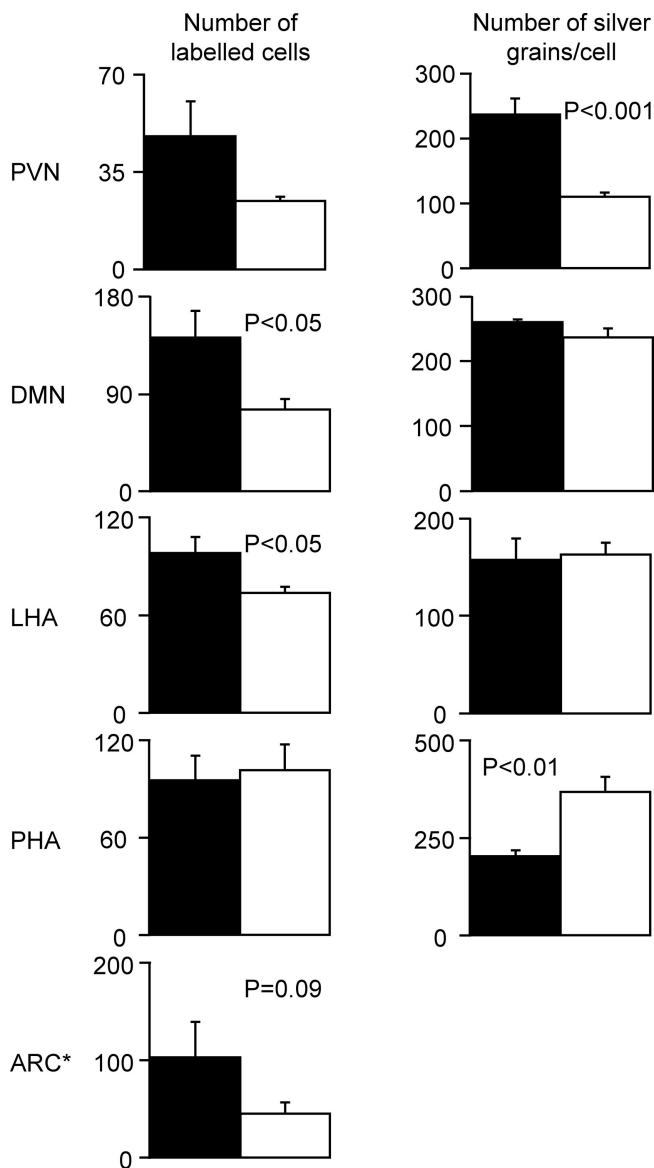
explained by the methodological differences (*i.e.* sex, diet and probes).

*Calcitonin gene-related peptide*

In short-term (10 days) ovx ewes, the calcitonin gene-related peptide (CGRP) is localised in hypothalamic magnocellular neurones, in the ventral paraventricular and supraoptic nuclei. Fewer CGRP-ir cell bodies are present in the preoptic area, and the anterior and basal hypothalamus (Herbison et al., 1993). In colchicine treated ewes, CGRP-ir neurones are observed in the same areas as previously described (personal observations, Fig. 9). No studies have been conducted concerning the role of CGRP in the regulation of nutrition. However, one study suggests a potential role in feeding behaviour. An icv injection of CGRP did not affect the first three-hour period of food intake, while a significant increase in daily food intake was observed (Bueno et al., 1986).

*Alpha-melanocyte-stimulating hormone*

The distribution of alpha-melanocyte-stimulating hormone (alpha-MSH) is similar to that of beta-End (Fig. 3, see above). High densities of alpha-MSH-ir neurones are found in the infundibular nucleus (Fig. 10a, b), the ventrolateral (Fig. 10c) and dorsolateral (Fig.

10d) hypothalamic area of sheep as well as other species (rat: Watson and Akil, 1979; Kachaturian et al., 1985; cat: Covenas et al., 1996).

In rodents, icv injection of alpha-MSH inhibits food intake (Table 1), whereas its central effect on food intake has not been determined in sheep.

The involvement of this peptide in nutrition is also indicated by the distribution of its receptors. The melanocortin receptor-3 (MC3-R) and -4 mRNA are observed in the anterior hypothalamus, in the paraventricular, dorsomedial, and ventromedial hypothalamic nuclei, and in the lateral hypothalamic area of intact adult ewes. In the arcuate nucleus only MC3-R mRNA are present (Iqbal et al., 2001b). These expressions are not modified by the long-term alteration of body weight in ovx ewes (Iqbal et al., 2001b).

In rodents, neurotensin (*NT*), glucagon-like peptide-1 (*GLP-1*) and thyrotropin-releasing hormone (*TRH*) are known to decrease food intake after icv administration. The organization of the NT- and GLP-1 neuronal systems has been described in the diencephalon (Antonopoulos et al., 1989a) and brainstem (Mercer et al., 1998), respectively, of adult sheep, but their roles on food intake are still unknown in sheep. In the diencephalon of foetal sheep, the highest densities of TRH-ir cells were observed in the organum vasculosum of the lamina terminalis and in the paraventricular
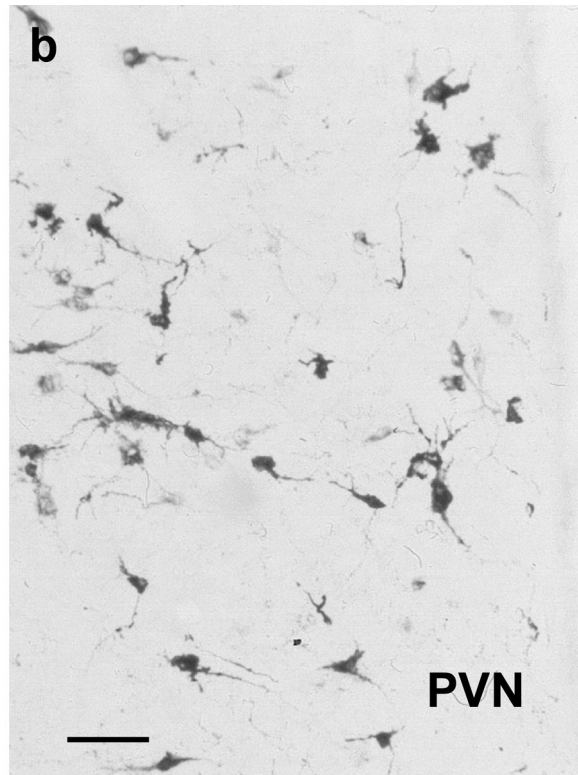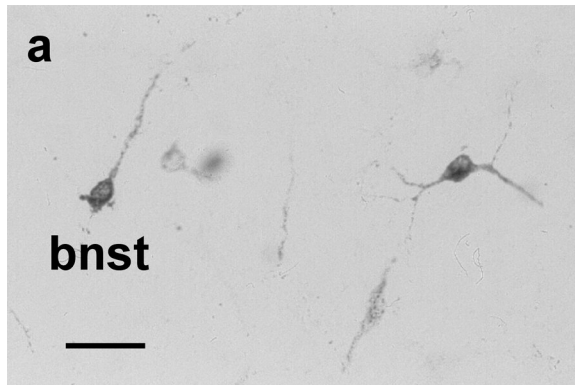
**Fig. 9.** CGRP-immunoreactive neurones observed in the bed nucleus of the stria terminalis **(a)** and paraventricular nucleus **(b)** of colchicine-treated adult ewes. (bnst, bed nucleus of the stria terminalis; III, third ventricle; PVN, paraventricular nucleus). Scale bar: a, 100 µm; b, 50 µm.

nucleus of the hypothalamus (McDonald et al., 1993). This latter structure contains the highest density of TRH-labelled neurones in adult colchicine-treated ewes (Fig. 11). Inhibitory effects on food intake in ovine have been described only for TRH (Ruckebush and Malbert, 1986).

## Hormonal regulatory systems implicated in nutritional regulation of hypothalamic neuropeptides

In addition to the role of hypothalamic neuropeptides in nutrition, we must consider their hormonal regulatory systems. The involvement of insulin, leptin and ghrelin, in feeding and nutrition regulations have been abundantly described in numerous species. These actions may be partly mediated by neuropeptides neuronal systems that present binding-site or receptors of these three hormones. In this section, we compare the major relevant data from rodents and sheep.

### Insulin

In rodents, insulin receptor mRNA are localized in the arcuate nucleus (Marks et al., 1990), and insulin binding sites are observed in the supraoptic and periventricular nuclei (Schulingkamp et al., 2000). In sheep, the distribution of insulin-binding sites in the brain has never been described.

Interactions between insulin and hypothalamic neuropeptides have been suggested in several studies. In rats, an icv injection of insulin reduces both galanin and
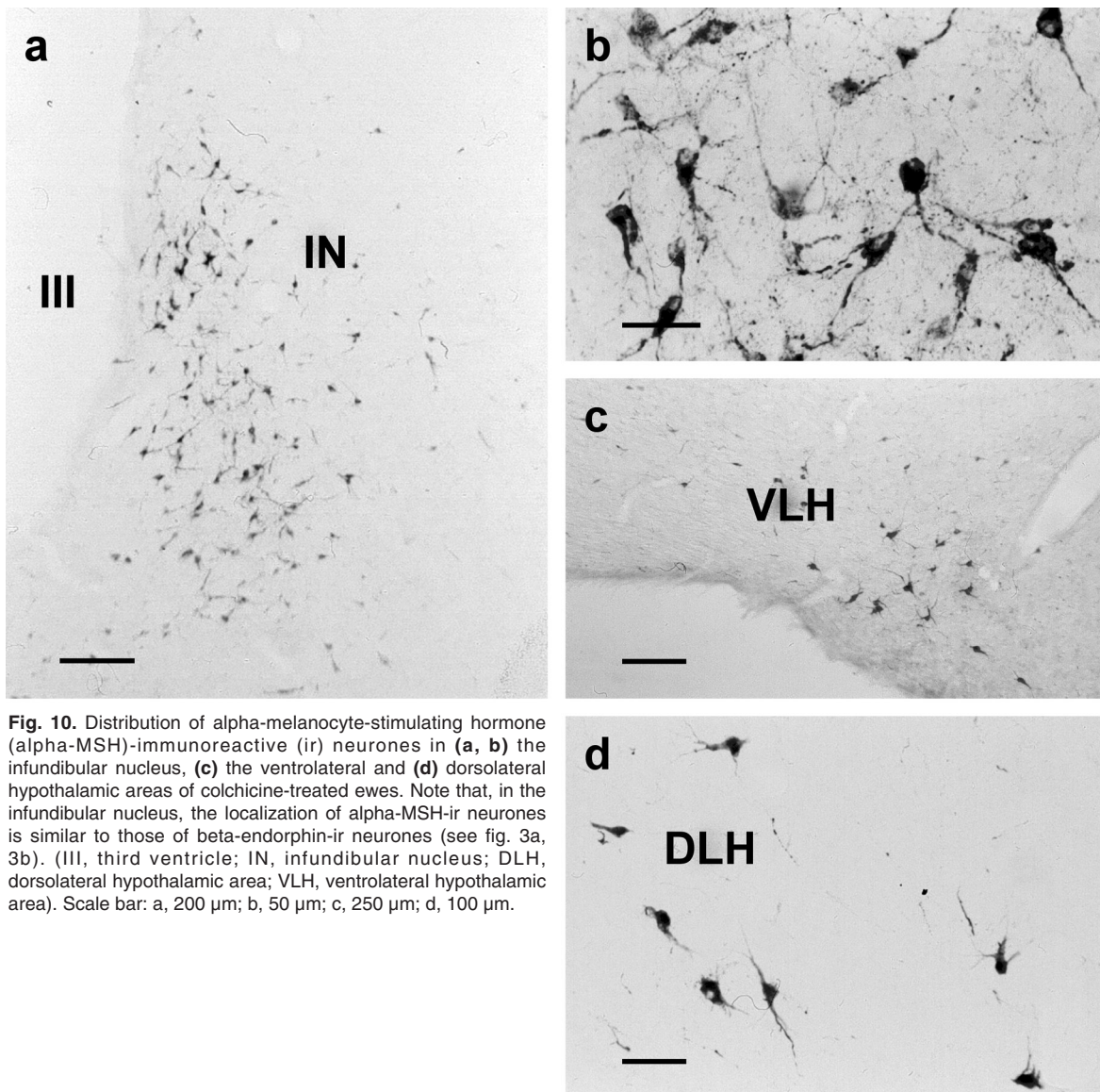
**Fig. 10.** Distribution of alpha-melanocyte-stimulating hormone (alpha-MSH)-immunoreactive (ir) neurones in **(a, b)** the infundibular nucleus, **(c)** the ventrolateral and **(d)** dorsolateral hypothalamic areas of colchicine-treated ewes. Note that, in the infundibular nucleus, the localization of alpha-MSH-ir neurones is similar to those of beta-endorphin-ir neurones (see fig. 3a, 3b). (III, third ventricle; IN, infundibular nucleus; DLH, dorsolateral hypothalamic area; VLH, ventrolateral hypothalamic area). Scale bar: a, 200 μm; b, 50 μm; c, 250 μm; d, 100 μm.

NPY gene expression and peptide immunoreactivity in several hypothalamic areas like arcuate and paraventricular nuclei (Wang and Leibowitz, 1997). Insulin injection leads to increased levels of MCH mRNA and MCH-ir staining in the lateral hypothalamus of rats (Bahjaoui-Bouhaddi et al., 1994). The level of orexin mRNA in the lateral hypothalamic area and the perifornical area of adult male rats, is increased after an intraperitoneal injection of insulin (Griffond et al., 1999). In rats, hypothalamic neurones co-express insulin receptor and POMC, and icv injections of insulin increase the expression of POMC mRNA in fasted animals (Benoit et al., 2002).

In sheep, no direct effect of insulin on neuropeptide expression has been described, but such an effect could be hypothesized since insulin level is sensitive to nutrition, and insulin injection modifies food intake. A high protein and energy diet causes an increased insulin level in plasma and cerebrospinal fluid of rams (Miller et al., 1998). A similar effect is observed in plasma of over-fed ovx adult ewes that present modifications of neuropeptide immunoreactivity (Chaillou et al., 2002a,b, 2003).

## Leptin

The distribution of the leptin receptor (Ob-R) has been described in sheep (Williams et al., 1999), with the highest density found in the hypothalamus. As in rodents (Håkansson et al., 1998), numerous neuropeptide-containing neurones are potential targets of leptin in the sheep hypothalamus (Table 2).

In rodents, Ob-R are localized on neuropeptide-containing neurones (Håkansson et al., 1998); leptin injection modifies some neuropeptide expression and interacts with their action on feeding behaviour. For example, icv injections of leptin, for 5-days, decreases the expression of galanin, MCH, POMC and NPY mRNA, and increases the expression of NT mRNA in male rats (Sahu, 1998a). In the same animal model, an icv injection of leptin decreases the stimulating central effect of MCH, galanin, or NPY on food intake (Sahu,

**Table 2.** Summary of the co-localizations of leptin-receptor and different neuropeptides in the sheep hypothalamus.

| NEUROPEPTIDE | LOCALIZATION | PROPORTION | ANIMAL MODEL | REFERENCES |
|---|---|---|---|---|
| CRH | Paraventricular nucleus | 30% | Adult castrated male | Iqbal et al., 2001a |
| Galanin | Arcuate nucleus | 60% | Adult castrated male | Iqbal et al., 2001a |
| MCH | Lateral hypothalamic area | 100% | Adult castrated male | Iqbal et al., 2001a |
| NPY | Arcuate nucleus | 100% | Adult ewes | Williams et al., 1999 |
|  |  | 60% | Adult castrated male | Iqbal et al., 2001a |
| Orexin | Lateral hypothalamic area | 100% | Adult castrated male | Iqbal et al., 2001a |
| POMC | Arcuate nucleus | 90% | Adult castrated male | Iqbal et al., 2001a |
| SRIF | Arcuate nucleus | 100% | Adult castrated male | Iqbal et al., 2000 |
|  | Dorsomedial and ventromedial parts of the hypothalamus | 100% | Adult castrated male | Iqbal et al., 2000 |
|  | Periventricular area (periventricular nucleus, anterior hypothalamic area and parvocellular part of the paraventricular nucleus) | 50% | Adult castrated male | Iqbal et al., 2000 |
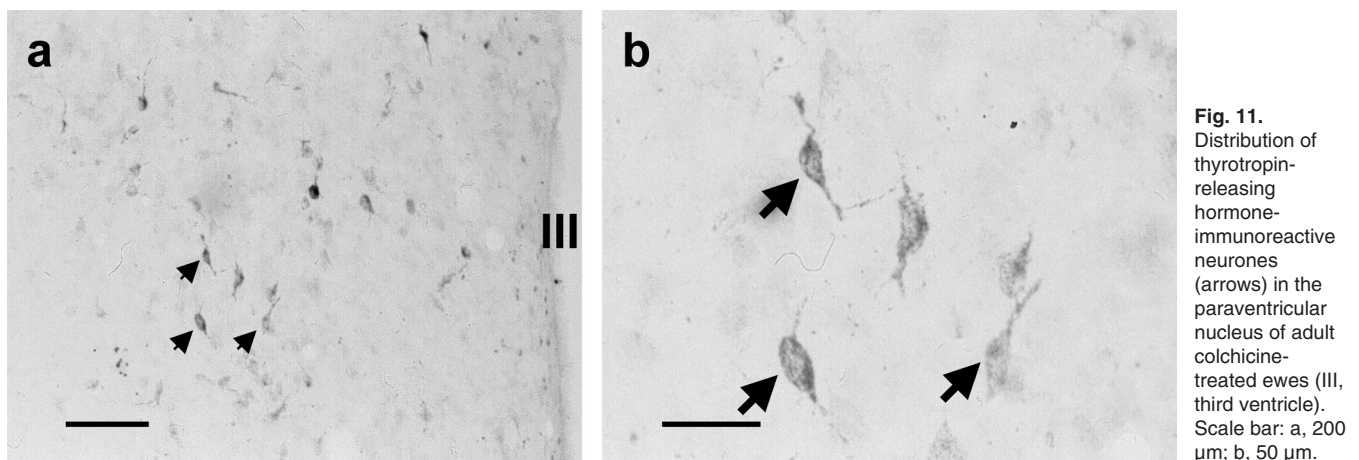


**Fig. 11.** Distribution of thyrotropin-releasing hormone-immunoreactive neurones (arrows) in the paraventricular nucleus of adult colchicine-treated ewes (III, third ventricle). Scale bar: a, 200 µm; b, 50 µm.

1998b). In mice, subcutaneous perfusion of leptin for 7-days causes an increase of MCH mRNA and MCH peptide levels in the hypothalamus/thalamus (Huang et al., 1999). In rats, the inhibitory effect of icv administration of leptin on food intake is substantially abolished by pre-treating animals with antibodies against CRH, but not urocortin (Okamoto et al., 2001).

All these data suggest that the variations in plasma levels of leptin that are related to nutrition, act on hypothalamic neuropeptides to regulate feeding behaviour and/or pituitary hormonal secretions.

No direct interacting effects of leptin and hypothalamic neuropeptides have been described for sheep nutritional regulations. Major differences exist between sheep and rodents, even if leptin and its receptor are sensitive to nutrition. The expression of Ob-R mRNA is higher in the ventromedial and arcuate nuclei of fed-restricted ovx ewes than in those of well-fed controls (Dyer et al., 1997). The same up-regulation is observed in the arcuate nucleus of adult male castrated sheep that have been fasted for 4-days (Adam et al., 2002). In some cases, increased Ob-R expression is associated with a decrease in leptin plasma level and an increase of NPY mRNA, or AGRP mRNA expression (Adam et al., 2002, Sorensen et al., 2002). However, in adult undernourished ovx ewes, modifications in the number of NPY-ir, galanin-ir, or CRH-ir neurones have been observed, with no effect on leptin plasma level (Chaillou et al., 2002a,b, 2003). Moreover, the central effects of leptin on food intake are variable and depend on criteria such as injection route, period of injection, sex, steroids, season or body score (Henry et al., 1999, 2001c; Blache et al., 2000; Clarke et al., 2000; Morrison et al., 2001, 2002). In ovines, the role of leptin as a key regulator in the nutritional modulation of hypothalamic neuropeptides remains to be demonstrated.

### Ghrelin

Ghrelin is an endogenous ligand for growth hormone secretagogue receptor, originally isolated from the stomach. This peptide has a central stimulatory effect on food intake in rats (Nakazato et al., 2001). The growth hormone secretagogue receptors are distributed throughout the brain, especially in the hypothalamus (supraoptic, ventromedial, arcuate, paraventricular, and tuberomammillary nuclei) (Guan et al., 1997), and their co-localization with several hypothalamic neuropeptides could mediate the actions of ghrelin on them (Tables 3a,b).

In the sheep abomasum, the ruminant secretor stomach, the presence of ghrelin has been demonstrated by immunohistochemistry in cells from the neck to the base of the oxyntic glands (Hayashida et al., 2001). In rams, plasma ghrelin levels increase just before feeding, tend to increase in pseudo-fed animals and decrease during feeding (Sugino et al., 2002a). These secretion profiles depend on the frequency of meal distribution (Sugino et al., 2002b).

In sheep, no data have been published on potential interactions between ghrelin secretions and hypothalamic neuropeptides as regards nutrition.

### Conclusion

This review considers most of the data concerning the hypothalamic neuropeptides and their implication in nutritional regulation in sheep. Compared to rodent, there have been fewer studies of neuropeptides such as AGRP, CART, CGRP, MCH, galanin, but the use of histochemistry allows description of their partial distribution and also their potential sensitivity to different nutritional conditions.

**Table 3.** Summary of **(a)** the co-localizations of growth hormone secretagogue-receptor mRNA and NPY, SRIF and POMC mRNA, and **(b)** the central ghrelin effects on NPY, AgRP, POMC and SRIF mRNA expression, in rats.

Table 3a

| NEUROPEPTIDE | LOCALIZATION | PROPORTION | REFERENCE |
|---|---|---|---|
| NPY | Arcuate nucleus | 90% | Willesen et al., 1999 |
| SRIF | Arcuate nucleus | 30% | Willesen et al., 1999 |
| POMC | Arcuate nucleus | 8% | Willesen et al., 1999 |

Table 3b

| NEUROPEPTIDE | LOCALIZATION | EFFECT | INJECTION MODALITIES |
|---|---|---|---|
| NPY mRNA | Hypothalamus | + | Acute icv injection (Shintani et al., 2001) |
| | Arcuate nucleus | + | Acute (Nakazato et al., 2001) or chronic (Kamegai et al., 2001a) icv injection |
| AGRP mRNA | Arcuate nucleus | + | Acute (Kamegai et al., 2001b) or chronic (Kamegai et al., 2001a) icv injection |
| POMC mRNA | Arcuate nucleus | = | Acute (Kamegai et al., 2001b) or chronic (Kamegai et al., 2001a) icv injection |
| SRIF mRNA | Arcuate nucleus | = | Acute (Kamegai et al., 2001b) or chronic (Kamegai et al., 2001a) icv injection |
| | Periventricular nucleus | = | Acute (Kamegai et al., 2001b) or chronic (Kamegai et al., 2001a) icv injection |

In rodents, different complementary methods have been used to study the role of hypothalamic neuropeptides in nutrition. The major methods include neuropeptide central injections, peptide and mRNA assays from microdissected samples, and functional neuroanatomy.

It is clear from our review that the results may differ according to the investigation methods and experimental protocols. Neuropeptide central injection is a good method to demonstrate an effect on feeding behaviour, but adequate knowledge of the neuronal system is necessary to choose a relevant injection site. For instance, the first studies on met-enkephalin were done with peptide assays of microdissected samples from the mediobasal hypothalamus, and the authors observed an increasing effect after starvation. The use of *in situ* hybridization demonstrated differential regulation by undernutrition between periventricular, paraventricular and ventromedial nuclei, all found in the mediobasal hypothalamic area. Among the different neuronal systems reviewed, only NPY neurones have sufficient variability to be observed with different methods. However, in the arcuate nucleus, the lateral and ventral subpopulations specifically sensitive to undernutrition and refeeding, respectively, have been identified only by immunohistochemistry. Neuroanatomical tools are necessary to identify accurately variations in, sometimes small, neuronal population in a heterogeneous structure like the brain.

The development of image analysis systems permits easy quantification of the signal obtained by *in situ* hybridization. With these tools the number of mRNA-containing neurones and silver grains per cell can be measured. The first component specifies the number of recruited cells, and the second gives information about the level of expression in each cell. With these two parameters, the sensitivity of neuropeptide-neuronal systems to undernutrition can be demonstrated. For instance, only the level of mRNA MCH expression is modified by undernutrition; the number of mRNA-containing neurones and MCH-ir neurones are unchanged. In the case of the CART neuronal system, undernutrition has distinct effects on the hypothalamic nucleus. Moreover, these effects concern only the number of recruited cells (dorsomedial nucleus, lateral hypothalamic area), mRNA expression in each cell (posterior hypothalamic area) or both (paraventricular nucleus).

The majority of the studies cited above used histochemistry to describe neuronal systems and demonstrate sensitivity to nutritional conditions. Immunohistochemitry and *in situ* hybridization provide complementary data concerning peptide accumulation and mRNA expression. They constitute the best methods to obtain good anatomical descriptions of an unknown neuronal system and to compare it across different experimental animal groups.

The experiments in sheep show that the peptidergic neurone systems involved in the regulation of nutrition are generally similar to those observed in other species, but they present specific differences. In addition, their regulation may also be different, as illustrated by sensitivity to leptin; this point is closely related to characteristics of the animal model. Overall, these studies provide new insights into the involvement of peptidergic neurones in the control of nutrition and energy balance in mammals.

## References

Adam C.L. and Findlay P.A. (1998). Inhibition of luteinizing hormone secretion and expression of c-fos and corticotrophin-releasing factor genes in the paraventricular nucleus during insulin-induced hypoglycemia in sheep. J. Neuroendocrinol. 10, 777-783.

Adam C.L., Findlay P.A., Kyle C.E., Young P. and Mercer J.G. (1997). Effect of chronic food restriction on pulsatile luteinizing hormone secretion and hypothalamic neuropeptide Y gene expression in castrate male sheep. J. Endocrinol. 152, 329-337.

Adam C.L., Archer Z.A., Findlay P.A., Thomas L. and Marie M. (2002). Hypothalamic gene expression in sheep for cocaine- and amphetamine-regulated transcript, pro-opiomelanocortin, neuropeptide Y, agouti-related peptide and leptin receptor and responses to negative energy balance. Neuroendocrinology 75, 250-256.

Antonopoulos J., Papadopoulos G.C., Karamanlidis A.N., Parnavelas J.G., Dinopoulos A. and Michaloudi H. (1987). VIP- and CCK-like-immunoreactive neurons in the hedgehog (*Erinaceus europaeus*) and sheep (*Ovis aries*) brain. J. Comp. Neurol. 263, 290-307.

Antonopoulos J., Karamanlidis A.N., Papadopoulos G.C., Michaloudi H., Dinopoulos A. and Parnavelas J.G. (1989a). Neuropeptide Y-like immunoreactive neurons in the hedgehog (*Erinaceus europaeus*) and the sheep (*Ovis aries*) brain. J. Hirnforsch. 30, 349-360.

Antonopoulos J., Papadopoulos G.C., Karamanlidis A.N. and Michaloudi H. (1989b). Distribution of neuropeptides in the infundibular nucleus of the sheep. Neuropeptides 14, 121-128.

Archer Z.A., Findlay P.A., Rhind S.M., Mercer J.G. and Adam C.L. (2002). Orexin gene expression and regulation by photoperiod in the sheep hypothalamus. Regul. Pept. 104, 41-45.

Bahjaoui-Bouhaddi M., Fellmann D., Griffond B. and Bugnon C. (1994). Insulin treatment stimulates the rat melanin-concentrating hormone-producing neurons. Neuropeptides 27, 251-258.

Baile C.A., McLaughlin C.L., Buonomo F.C., Lauterio T.J., Marson L. and Della-Fera M.A. (1987). Opioid peptides and the control of feeding in sheep. Federation Proc. 46, 173-177.

Barker-Gibb M.L. and Clarke I.J. (1996). Increased galanin and neuropeptide-Y immunoreactivity within the hypothalamus of ovariectomised ewes following a prolonged period of reduced body weight is associated with changes in plasma growth hormone but not gonadotropin levels. Neuroendocrinology 64, 194-207.

Beck B., Jhanwar-Uniyal M., Burlet A., Chapleur-Chateau M., Leibowitz S.F. and Burlet C. (1990). Rapid and localized alterations of neuropeptide Y in discrete hypothalamic nuclei with feeding status. Brain Res. 528, 245-249.

Benoit S.C., Air E.L., Coolen L.M., Strauss R., Jackman A., Clegg D.J.,

Seeley R.J. and Woods S.C. (2002). The catabolic action of insulin in the brain is mediated by melanocortins. J. Neurosci. 22, 9048-9052.

Blache D., Celi P., Blackberry M.A., Dynes R.A. and Martin G.B. (2000). Decrease in voluntary feed intake and pulsatile luteinizing hormone secretion after intracerebroventricular infusion of recombinant bovine leptin in mature male sheep. Reprod. Fertil. Dev. 12, 373-381.

Bruneau G. and Tillet Y. (1998). Localization of the preprosomatostatin-mRNA by in situ hybridization in the ewe hypothalamus. Peptides 19, 1749-1758.

Bueno L., Fargeas M.J. and Julie P. (1986). Effects of calcitonin and CGRP alone or in combination on food intake and forestomach (reticulum) motility in sheep. Physiol. Behav. 36, 907-911.

Cepoi D., Sutton S., Arias C., Sawchenko P. and Vale W.W. (1999). Ovine genomic urocortin: cloning, pharmacologic characterization, and distribution of central mRNA. Mol. Brain Res. 68, 109-118.

Chaillou E., Tramu G., Thibault J. and Tillet Y. (1998). Presence of galanin in dopaminergic neurons of the sheep infundibular nucleus: a double staining immunohisochemical study. J. Chem. Neuroanat. 15, 251-259.

Chaillou E., Tramu G. and Tillet Y. (1999). Distribution of galanin immunoreactivity in the sheep diencephalon. J. Chem. Neuroanat. 17, 129-146.

Chaillou E., Baumont R., Tramu G. and Tillet Y. (2000). Effect of feeding on Fos protein expression in sheep hypothalamus with special reference to the supraoptic and paraventricular nuclei: an immunohistochemical study. Eur. J. Neurosci. 12, 4515-4524.

Chaillou E., Baumont R., Chilliard Y. and Tillet Y. (2002a). Several subpopulations of neuropeptide Y-containing neurons exist in the infundibular nucleus of sheep: an immunohistochemical study of animals on different diets. J. Comp. Neurol. 444, 129-143.

Chaillou E., Baumont R., Tramu G. and Tillet Y. (2002b). Long-term undernutrition followed by short-term refeeding effects on the corticotropin-releasing hormone containing neurones in the paraventricular nucleus: an immunohistochemical study in sheep. J. Neuroendocrinol. 14, 269-275.

Chaillou E., Baumont R., Fellmann D., Tramu G. and Tillet Y. (2003). Sensitivity of galanin- and melanin-concentrating hormone-containing neurones to nutritional status: an immunohistochemical study in the ovariectomized ewe. J. Neuroendocrinol. 15, 459-467.

Clarke I.J., Tilbrook A.J., Turner A.I., Doughton B.W. and Goding J.W. (2000). Sex, fat and the tilt of the earth: effects of sex and season on the feeding response to centrally administered leptin in sheep. Endocrinology 142, 2725-2728.

Chronwall B.M., DiMaggio D.A., Massari V.J., Pickel V.M., Ruggiero D.A. and O'Donohue T.L. (1985). The anatomy of neuropeptide-Y-containing neurons in the rat brain. Neuroscience 15, 1159-1181.

Covenas R., De Leon M., Narvaez J.A., Tramu G., Aguirre J.A. and Gonzalez-Baron S. (1996). Mapping of alpha-melanocyte-stimulating hormone-like immunoreactivity in the cat diencephalon. Peptides 17, 845-852.

Della-Fera M.A. and Baile C.A. (1979). CCK-octapeptide injected in CSF causes satiety in sheep. Ann. Rech. Vét. 10, 234-236.

Della-Fera M.A. and Baile C.A. (1981). Cholecystokinin antibody injected in cerebral ventricules stimulates feeding in sheep. Science 212, 687-689.

Dyer C.J., Simmons J.M., Matteri R.L. and Keisler D.H. (1997). Leptin receptor mRNA is expressed in ewe anterior pituitary and adipose tissues and is differentially expressed in hypothalamic regions of well-fed and feed-restricted ewes. Domest. Anim. Endocrinol. 14, 119-128.

Edwards C.M.B., Abusnana S., Sunter D., Murphy K.G., Ghatei M.A. and Bloom S.R. (1999). The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. J. Endocrinol. 160, R7-R12.

Engler D., Pham T., Fullerton M.J., Ooi G., Funder J.W. and Clarke I.J. (1989). Studies of the secretion of corticotropin-releasing factor and arginine vasopressin into the hypophysial-portal circulation of the conscious sheep. I. Effect of an audiovisual stimulus and insulin-induced hypoglycemia. Neuroendocrinology 49, 367-381.

Griffond B., Risold P.-Y., Jacquemard C., Colard C. and Fellmann D. (1999). Insulin-induced hypoglycemia increases preprohypocretin (orexin) mRNA in the rat lateral hypothalamic area. Neurosci. Lett. 262, 77-80.

Guan X.-M., Yu H., Palyha O.C., McKee K.K., Feighner S.D., Sirinathsinghji D.J.S., Smith R.G., Van der Ploeg L.H.T. and Howard A.D. (1997). Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. Mol. Brain Res. 48, 23-29.

Håkansson M.-L., Brown H., Ghilardi N., Skoda R.C. and Meister B. (1998). Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. J. Neurosci. 18, 559-572.

Hayashida T., Murakami K., Mogi K., Nishihara M., Nakazato M., Mondal M.S., Horii Y., Kojima M., Kangawa K., Murakami N. (2001). Ghrelin in domestic animals: distribution in stomach and its possible role. Domest. Anim. Endocrinol. 21, 17-24.

Henry B.A., Goding J.W., Alexander W.S., Tilbrook A.J., Canny B.J., Dunshea F., Rao A., Mansell A. and Clarke I.J. (1999). Central administration of leptin to ovariectomized ewes inhibits food intake without affecting the secretion of hormones from the pituitary gland: evidence for a dissociation of effects on appetite and neuroendocrine function. Endocrinology 140, 1175-1182.

Henry B.A., Tilbrook A.J., Dunshea F.R., Rao A., Blache D., Martin G.B. and Clarke I.J. (2000). Long-term alterations in adiposity affect the expression of melanin-concentrating hormone and enkephalin but not proopiomelanocortin in the hypothalamus of ovariectomized ewes. Endocrinology 141, 1506-1514.

Henry B.A., Rao A., Ikenasio B.A., Mountjoy K.G., Tilbrook A.J. and Clarke I.J. (2001a). Differential expression of cocaine- and amphetamine-regulated transcript and agouti related-protein in chronically food-restricted sheep. Brain Res. 918, 40-50.

Henry B.A., Rao A., Tilbrook A.J. and Clarke I.J. (2001b). Chronic food-restriction alters the expression of somatostatin and growth hormone-releasing hormone in the ovariectomised ewe. J. Endocrinol. 170, R1-R6.

Henry B.A., Goding J.W., Tilbrook A.J., Dunshea F.R. and Clarke I.J. (2001c). Intracerebroventricular infusion of leptin elevates the secretion of luteinising hormone without affecting food intake in long-term food-restricted sheep, but increases growth hormone irrespective of bodyweight. J. Endocrinol. 168, 67-77.

Herbison A.E., Robinson J.E. and Skinner D.C. (1993). Calcitonin gene-related peptide (CGRP): immunocytochemical identification of a neuropeptide synthesised by ventral paraventricular magnocellular neurones in the sheep. Brain Res. 611, 147-151.

Holmberg B.J. and Malven P.V. (1997). Neural levels of cholecystokinin peptide and mRNA during dietary stimulation of growth and LH secretion in growth-retarded lambs. Dom. Anim. Endocrinol. 14, 304-

315.

Holmberg B.J., Morrison C.D. and Keisler D.H. (2001). Endocrine responses of ovariectomized ewes to i.c.v. infusion of urocortin. J. Endocrinol. 171, 517-524.

Huang Q., Viale A., Picard F., Nahon J. and Richard D. (1999). Effects of leptin on melanin-concentrating hormone expression in the brain of lean and obese Lep(ob)/Lep(ob) mice. Neuroendocrinology 69, 145-153.

Iqbal J., Pompolo S., Murakami T. and Clarke I.J. (2000). Localization of long-form leptin receptor in the somatostatin-containing neurons in the sheep hypothalamus. Brain Res. 887, 1-6.

Iqbal J., Pompolo S., Murakami T., Grouzmann E., Sakurai T., Meister B. and Clarke I.J. (2001a). Immunohistochemical characterization of localization of long-form leptin receptor (OB-Rb) in neurochemically defined cells in the ovine hypothalamus. Brain Res. 920, 55-64.

Iqbal J., Pompolo S., Dumont L.M., Wu C.S., Mountjoy K.G., Henry B.A. and Clarke I.J. (2001b). Long-term alterations in body weight do not affect the expression of melanocortin receptor-3 and –4 mRNA in the ovine hypothalamus. Neuroscience 105, 931-940.

Iqbal J., Henry B.A., Pompolo S., Rao A. and Clarke I.J. (2003). Long-term alteration in body-weight and food restriction does not affect the gene expression of either preproorexin or prodynorphin in the sheep. Neuroscience 118, 216-226.

Kalra S.P., Dube M.G., Sahu A., Phelps C.P. and Kalra P.S. (1991). Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. Proc. Natl. Acad. Sci. USA 88, 10931-10935.

Kamegai J., Tamura H., Shimizu T., Ishii S., Sugihara H. and Wakabayashi I. (2001a). Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and agouti-related protein mRNA levels and body weight in rats. Diabetes 50, 2438-2443.

Kamegai J., Tamura H., Shimizu T., Ishii S., Sugihara H. and Wakabayashi I. (2001b). Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. Endocrinology 141, 4797-4800.

Khachaturian H., Lewis M.E., Tsou K. and Watson S.J. (1985). Beta-endorphin, alpha-MSH, ACTH and related peptides. In: Handbook of chemical neuroanatomy, Vol. 4: GABA and neuropeptides in the CNS. Part I. Björklund A. and Hökfelt T. (eds). Elsevier Science Publishers B.V. Amsterdam, New York, Oxford. pp 216-272.

Kolodziejczyk E., Baertschi A.J. and Tramu G. (1983). Corticoliberin-immunoreactive cell bodies localised in two distinct areas of the sheep hypothalamus. Neuroscience 9, 261-270.

Marks J.L., Porte D., Stahl W.L. and Baskin D.G. (1990). Localization of insulin receptor mRNA in rat brain by in situ hybridization. Endocrinology 127, 3234-3236.

Marson L., Lauterio T.J., Della-Fera M.-A. and Baile C.A. (1987). Immunohistochemical distribution of cholecystokinin, dynorphin A and met-enkephalin neurons in sheep hypothalamus. Neuroscience Lett. 81, 35-40.

Matthews S.G., Heavens R.P. and Sirinathsinghji D.J.S. (1992). Distribution and cellular localization of preproenkephalin mRNA in the ovine brain and pituitary. Mol. Brain Res. 12, 349-355.

McDonald T.J., Hoffman G.E. and Nathanielsz P.W. (1993). Failure of bilateral paraventricular nuclear lesions to cause hypothalamic hypothyroidism in fetal sheep. Endocrinology 132, 371-376.

McShane T.M., Petersen S.L., McCrone S. and Keisler D.H. (1993). Influence of food restriction on neuropeptide-Y, proopiomelanocortin, and luteinizing hormone-releasing hormone

gene expression in sheep hypothalami. Biol. Reprod. 49, 831-839.

Mercer J.G., Moar K.M., Findlay P.A., Hoggard N. and Adam C.L. (1998). Association of leptin receptor (Ob-Rb), NPY and GLP-1 gene expression in the ovine and murine brainstem. Regul. Pept. 75-76, 271-278.

Miller D.W., Blache D., Boukhliq R., Curlewis J.D. and Martin G.B. (1998). Central metabolic messengers and the effects of nutrition on gonadotrophin secretion in sheep. J. Reprod. Fertil. 112, 347-356.

Miner J.L., Della-Fera M.A., Paterson J.A. and Baile C.A. (1989). Lateral cerebroventricular injection of neuropeptide Y stimulates feeding in sheep. Am. J. Physiol. 257, R383-R387.

Miner J.L., Della-Fera M.A. and Paterson J.A. (1990). Blockade of satiety factors by central injection of neuropeptide Y in sheep. J. Anim. Sci. 68, 3805-3811.

Morrison C.D., Daniel J.A., Holmberg B.J., Djiane J., Raver N., Gertler A. and Keisler D.H. (2001). Central infusion of leptin into well-fed and undernourished ewe lambs: effects on feed intake and serum concentrations of growth hormone and luteinizing hormone. J. Endocrinol. 168: 317-324.

Morrison C.D., Wood R., McFadin E.L., Whitley N.C. and Keisler D.H. (2002). Effect of intravenous infusion of recombinant ovine leptin on feed intake and serum concentrations of GH, LH, insulin, IGF-1, cortisol and thyroxine in growing prepubertal ewe lambs. Domest. Anim. Endocrinol. 22, 103-112.

Nakagawa Y., Shiosaka S., Emson P.C. and Tohyama M. (1985). Distribution of neuropeptide Y in the forebrain and diencephalon: an immunohistochemical analysis. Brain Res. 361, 52-60.

Nakazato M., Murakami N., Date Y., Kojima M., Matsuo H. Kangawa K. and Matsukura S. (2001). A role for ghrelin in the central regulation of feeding. Nature 409, 194-198.

Ober J.A. and Malven P.V. (1992). Effect of growth retardation on pituitary luteinizing hormone and hypothalamic neuropeptide Y in ovariectomized sheep. Neuroendocrinology 56, 331-339.

Okamoto S., Kimura K. and Saito M. (2001). Anorectic effect of leptin is mediated by hypothalamic corticotropin-releasing hormone, but not by urocortin, in rats. Neurosci. Lett. 307, 179-182.

Palkovits M., Brownstein M.J. and Vale W. (1983). Corticotropin releasing factor (CRF) immunoreactivity in hypothalamic and extrahypothalamic nuclei of sheep brain. Neuroendocrinology 37, 302-305.

Papadopoulos G.C., Karamanlidis A.N., Dinopoulos A. and Antonopoulos J. (1986). Somatostatinlike immunoreactive neurons in the hedgehog (Erinaceus europaeus) and the sheep (*Ovis aries*) central nervous system. J. Comp. Neurol. 244, 174-192.

Parkes D.G. (1996). Diuretic and natriuretic actions of melanin concentrating hormone in conscious sheep. J. Neuroendocrinol. 8, 57-63.

Paull W.K., Schöler J., Arimura A., Meyers C.A., Chang J.K., Chang D. and Shimizu M. (1982). Immunocytochemical localization of CRF in the ovine hypothalamus. Peptides 1, 183-191.

Polkowska J. and Gladysz A. (2001). Effect of food manipulation on the neuropeptide Y neuronal system in the diencephalon of ewes. J. Chem. Neuroanat. 21, 149-159.

Prasad B.M., Conover C.D., Sarkar D.K., Rabii J. and Advis J.-P. (1993). Feed restriction in prepubertal lambs: effect on puberty onset and on in vivo release of luteinizing-hormone-releasing hormone, neuropeptide Y and beta-endorphin from the posterior-lateral median eminence. Neuroendocrinology 57, 1171-1181.

Ruckebusch Y. and Malbert C.H. (1986). Stimulation and inhibition of

food intake in sheep by centrally-administered hypothalamic releasing factors. Life Sci. 38, 929-934.

Sahu A. (1998a). Evidence suggesting that galanin (GAL), melanin-concentrating hormone (MCH), neurotensin (NT), proopiomelanocortin (POMC) and neuropeptide Y (NPY) are targets of leptin signaling in the hypothalamus. Endocrinology 139, 795-798.

Sahu A. (1998b). Leptin decreases food intake induced by melanin-concentrating hormone (MCH), galanin (GAL) and neuropeptide Y (NPY) in the rat. Endocrinology 139, 4739-4742.

Sahu A., Kalra P.S. and Kalra S.P. (1988). Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. Peptides 9, 83-86.

Sartin J.L., Dyer C., Matteri R., Buxton D., Buonomo F., Shores M., Baker J., Osborne J.A., Braden T. and Steele B. (2001). Effect of intracerebroventricular orexin-B on food intake in sheep. J. Anim. Sci. 79, 1573-1577.

Scallet A.C., Della-Fera M.A. and Baile C.A. (1985). Satiety, hunger and regional brain content of cholecystokinin/gastrin and met-enkephalin immunoreactivity in sheep. Peptides 6, 937-943.

Schulingkamp R.J., Pagano T.C., Hung D. and Raffa R.B. (2000). Insulin receptors and insulin action in the brain: review and clinical implications. Neurosci. Behav. Rev. 24, 855-872.

Shintani M., Ogawa Y., Ebihara K., Aizawa-Abe M., Miyanaga F., Takaya K., Hayashi T., Inoue G., Hosoda K., Kojima M., Kangawa K. and Nakao K. (2001). Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes laptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes 50, 227-232.

Silva R.M., Hadjimarkou M.M., Rossi G.C., Pasternak G.W. and Bodnar R.J. (2001). ß-endorphin-induced feeding: pharmacological characterization using selective opioid antagonists and antisense probes in rats. J. Pharmacol. Experiment. Therapeutics 297, 590-596.

Smith G.P. (1999). Introduction to the reviews on peptides and the control of food intake and body weight. Neuropeptides 33, 323-328.

Sorensen A., Adam C.L., Findlay P.A., Marie M., Thomas L., Travers M.T. and Vernon R.G. (2002). Leptin secretion and hypothalamic neuropeptide and receptor gene expression in sheep. Am. J. Physiol. 282, R1227-R1235.

Spencer G.S.G. and Fadlalla A.M. (1989). Effect of intracerebroventricular administration of anti-somatostatin serum on the regulation of appetite in sheep. In: Endocrinology of farm animals. Boda K (ed). Kosice. Slovak Academy of Sciences. pp 51-54.

Sugino T., Hasegawa Y., Kikkawa Y., Yamaura J., Yamagishi M., Kurose Y., Kojima M., Kangawa K., Terashima Y. (2002a). A transient ghrelin surge occurs just before feeding in a scheduled meal-fed sheep. Biochem. Biophys. Res. Commun. 295, 255-260.

Sugino T., Yamaura J., Yamagishi M., Ogura A., Hayashi R., Kurose Y., Kojima M., Kangawa K., Hasegawa Y., Terashima Y. (2002b). A transient surge of ghrelin secretion before feeding is modified by different feeding regimens in sheep. Biochem. Biophys. Res. Commun. 298, 785-788.

Sunagawa K., Weisiger R.S., McKinley M.J., Purcell B.S., Thomson C.

and Burns P.L. (2000). The role of corticotropin-releasing factor and urocortin in brain mechanisms controlling feed intake of sheep. Asian-Aus. J. Anim. Sci. 13, 1529-1535.

Sunagawa K., Weisiger R.S., McKinley M.J., Purcell B.S., Thomson C. and Burns P.L. (2001). The role of neuropeptide Y in the central regulation of grass intake in sheep. Asian-Aust. J. Anim. Sci. 14, 35-40.

Tempel D.L., Leibowitz K.J. and Leibowitz S.F. (1988). Effects of PVN galanin on macronutrient selection. Peptides 9, 309-314.

Thomas G.B., Cummins J.T., Francis H., Sudbury A.W., McCloud P.I. and Clarke I.J. (1991). Effect of restricted feeding on the relationship between hypophysial portal concentrations of growth hormone (GH)-releasing factor and somatostatin, and jugular concentrations of GH in ovariectomized ewes. Endocrinology 128, 1151-1158.

Tillet Y. (1995). Distribution of neurotransmitters in the sheep brain. J. Reprod. Fertil. 49, 199-220.

Tillet Y., Batailler M. and Fellmann D. (1996). Distribution of melanin-concentrating hormone (MCH)-like immunoreactivity in neurons of the diencephalon of sheep. J. Chem. Neuroanat. 12, 135-145.

Vanderhaegen J.J. (1985). Neuronal choleystokinin. In: Handbook of chemical neuroanatomy, Vol. 4: GABA and neuropeptides in the CNS. Part I. Björklund A. and Hökfelt T.(eds). Elsevier Science Publishers B.V. Amsterdam, New York, Oxford. pp 406-435.

Wang J. and Leibowitz K.L. (1997). Central insulin inhibits hypothalamic and neuropeptide Y gene expression and peptide release in intact rats. Brain Res. 777, 231-236.

Watson S.J. and Akil H. (1979). The presence of two alpha-MSH positive cell groups in rat hypothalamus. Eur. J. Pharmacol. 58, 101-103.

Weisinger R.S., Blair-West J.R., Burns P., Denton D.A., McKinley M.J., Purcell B., Vale W., Rivier J. and Sunagawa K. (2000). The inhibitory effect of hormones associated with stress on Na appetite of sheep. Proc. Natl. Acad. Sci. USA 97, 2922-2927.

Whisnant C.S., Curto K. and Goodman R.L. (1992). Immunocytochemical localization of beta endorphin and gonadal steroid regulation of proopiomelanocortin messenger ribonucleic acid in the ewe. Neuroendocrinology 56, 812-821.

Whitlock B.K., Daniel J.A., McMahon C.D., Buonomo F.C., Wagner C.G., Steele B., and Sartin J.L. (2005). Intracerebroventricular melanin-concentrating hormone stimulates food intake in sheep. Dom. Anim. Endocrinol. 28, 224-232.

Willesen M.G., Kristensen P. and Romer J. (1999). Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. Neuroendocrinology 70, 306-316.

Williams L.M., Adam C.L., Mercer J.G., Moar K.M., Slater D., Hunter L., Findlay P.A. and Hoggard N. (1999). Leptin receptor and neuropeptide Y gene expression in the sheep brain. J. Neuroendocrinol. 11, 165-169.

Willoughby J.O., Oliver J.R., Fletcher T.P. and Clarke I.J. (1995). Distribution of somatostatin immunoreactivity in sheep hypothalamus: a comparison with that of the rat. Arch. Histol. Cytol. 58, 31-36.