Alterations of somatotrophic function in prion disease in sheep

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Abstract

This study aimed at investigating the possible linkage between natural scrapie and alterations of the somatotrophic axis. Scrapie-affected ewes exhibited 2-fold higher mean GH concentrations during both autumn and spring. GH pulse frequencies were higher in scrapie-affected ewes than in control animals (mean $\pm$ S.E.M. number of pulses/24 h: 10.4 ± 0.9 and 7.6 ± 0.9 for scrapie-affected and control ewes respectively) suggesting the involvement of central mechanisms. GH secretion induced by administration of an α_2 -adrenergic agonist, which acts centrally to stimulate GH secretion, was similar between healthy and scrapie-affected ewes (ratios of the area under the curve (AUC) of GH concentration after to the GH AUC before the agonist administration were 3.6 ± 1.6 and 4.9 ± 1.0 for

scrapie-affected and control ewes respectively). Finally, humoral markers and parameters of the metabolic status were determined to test the hypothesis that scrapie-associated alterations of GH secretion could be related to disruption of metabolic homeostasis. Glucose, insulin and urea plasma concentrations were higher in scrapie-affected than in healthy ewes. Neither leptin nor IGF-I levels were affected by scrapie. Total thyroxine (T4) was decreased in scrapie-affected ewes but free T4 and total and free tri-iodothyronine were not modified. In conclusion, our results showed the existence in scrapie-affected ewes of endocrine and metabolic alterations typical of acute illness proceeding, at least in part, from central mechanisms.

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Introduction

Multiple endocrine alterations are common features of several neurodegenerative disorders including prion-associated diseases (Carp *et al.* 1989, Ye & Carp 1996, Picard-Hagen *et al.* 1998, Busiguina *et al.* 2000, Ferrari *et al.* 2000, Gayrard *et al.* 2000). Despite the critical role of several endocrine systems in the maintenance of brain homeostasis and/or neuroprotective mechanisms, there has been little emphasis on the study of endocrine disorders in prion-related disease.

Prion diseases, and in particular scrapie, are chronic diseases often associated during their clinical stage with a wasting syndrome. One could expect such a disorder to arouse a full panel of adaptative mechanisms aimed at maintaining metabolic homeostasis. The somatotrophic axis is a key endocrine system for metabolic homeostasis. It is therefore very likely that prion disease can be associated with modification of the somatotrophic function.

Furthermore, several studies have suggested the existence of functional links between prion protein and the

somatotropic axis at both the level of growth hormone (GH) and insulin-like growth factor-I (IGF-I) secretions and/or actions (Lasmézas *et al.* 1993, Castelnau *et al.* 1994, Satoh *et al.* 2000, Östlund *et al.* 2001a). This lays the foundation for putative alterations of the somatotrophic function in disease associated with pathological modifications of the prion protein such as spongiform encephalopathy (Bounias & Purdey 2002). Moreover, the somatotrophic axis is deeply involved in neuroprotective mechanisms (Dore *et al.* 1997, Lackey *et al.* 2000). Thus, prion-related alteration of somatotrophic function could play a critical role in the physiopathology of the disease through a lack of neuroprotective mechanisms.

Collectively, all the above considerations prompted us to investigate the somatotrophic axis in sheep suffering from scrapie, a natural prion disease. We hypothesised that scrapie is associated with alterations of the somatotrophic axis resulting from central mechanisms. To test this hypothesis, we investigated GH secretion as the hub component of the somatotrophic axis lying at the interface between central and peripheral regulations. In relation to

our general hypothesis, we investigated the implication of three major components of the regulation of somatotrophic function, which all involve central mechanisms: (i) generation of GH pulsatility; (ii) α -adrenergic control; and (iii) metabolic regulation of GH secretion. With regard to the α -adrenergic control of GH secretion, we more particularly investigated responsiveness to an α_2 -adrenergic stimulus. Finally, we sought to determine whether clinical scrapie is associated with disruptions of metabolic homeostasis that could be related to perturbations of somatotrophic function.

Materials and Methods

General

Experiments were performed in adult ovary-intact ewes and intact rams. These were allocated to healthy or scrapie-affected groups on the basis of unambiguous clinical signs (i.e. pruritis, tremor, ataxia, behavioural troubles). Working in field conditions with a natural disease rendered it almost impossible to date the beginning of the disease. However, all the scrapie-affected animals included in the study showed well-established clinical signs for several weeks which means that they were studied within the 6 months preceding their death. Animals were maintained under natural photoperiod and were fed hay *ad libitum* and concentrates and had free access to water. At least 1 week before the beginning of experiments involving serial sampling, the animals were placed in metabolic cages in such a way that they could see each other. Twenty-four hours before the beginning of each sampling session, an indwelling venous catheter was aseptically placed in one jugular vein of each animal. Blood was collected either on lithium heparinate (for GH assay) or EDTA (for leptin, glucose, non-esterified fatty acids (NEFA), insulin, urea and β -hydroxybutyrate assays) or in dry tubes (IGF-I assay). The natural light/dark cycle was disrupted during the 24 h bleeding sessions as the lights remained on throughout sampling.

All procedures involving animals were performed in accordance with the French legal requirements regarding the protection of laboratory animals and with the authorisation for animal experimentation no. 001889 of the French Ministry of Agriculture.

Objective 1: Characterisation of scrapie-associated alterations of somatotrophic function: modification of mean GH concentration

Serial blood samples were collected as described above. GH secretion in sheep fluctuates with season (Barenton *et al.* 1987, 1988), so this experiment was performed during two different seasons. A first set of Manech Red Head (MRH) adult ewes ($n=7$ in both scrapie-affected and control groups) was sampled every hour for 24 h

during the anoestrous season (June) and a second set ($n=7$ and 6 for the scrapie-affected and control groups respectively) was sampled every hour for 10 h (0900–1900 h) during the breeding season (November). Three scrapie-affected and four healthy Romanov rams were sampled every 15 min for 6 h during the non-breeding season (March) to check that increased GH secretion could be observed in scrapie-affected animals of both sexes.

All the experiments in this study were performed on naturally occurring scrapie in sheep collected directly from farms. Under such conditions, it was very difficult to obtain precise information about the age of animals. It was therefore not possible to balance groups for age in our experimental trials. As GH secretion fluctuates with age in sheep, as in other species (Falconer *et al.* 1979, Iranmanesh *et al.* 1991), an epidemiological survey was performed to assess the potential impact of age on the GH differences observed between healthy and scrapie-affected sheep. A single blood sample was collected at random from different populations of healthy and scrapie-affected ewes for GH assay. Data were obtained from 47 healthy ewes and 62 scrapie-affected ewes during the clinical stage of the disease. Scrapie diagnosis was confirmed post mortem in all the scrapie-affected ewes by histological examination of the brain.

Objective 2: Are the effects of scrapie on mean GH concentration related to alteration of pulsatility?

This experiment was performed in the middle of the anoestrous season (May) on seven healthy and seven scrapie-affected adult MRH ewes. The animals received a concentrate meal twice a day (200 g between 0900 and 1000 h and 200 g between 1600 and 1700 h) and had water and hay available *ad libitum*. Blood samples were collected every 10 min for 24 h. One of every three samples was assayed for GH. One control ewe became sick shortly after this sampling period and was not included in the subsequent sampling period to characterise metabolic status (objective 4).

Objective 3: Are the effects of scrapie on mean GH concentration related to responsiveness to α_2 -adrenergic stimulation?

Blood samples were obtained from four healthy and four scrapie-affected ewes for 3 h before and 3 h after an i.v. injection of an α_2 -adrenergic agonist (Romifidin Sedivet, Boehringer-Ingelheim, 51060 Reins, France; 0.05 mg/kg). Blood was collected every 15 min during the control period and 1, 2, 4, 8, 15 and thereafter every 15 min after Romifidin administration. As no changes in GH concentration were observed during the 8 min following the agonist injection, only samples collected at 15 min intervals were taken into account for the final analysis.

Objective 4: Is scrapie associated with metabolic disturbances?

In a first experiment, animals of the GH pulsatility study (objective 2) were used to determine parameters and humoral markers of the metabolic status. One blood sample was collected just before (i.e. at least 12 h after distribution of the previous evening meal) and 4 h after distribution of the morning concentrate meal from the six remaining control and the seven scrapie-affected ewes. These samples were used for the measurement of metabolic parameters (urea, glycaemia, plasma β -hydroxybutyrate, non-esterified fatty acids (NEFA) and humoral markers of metabolic homeostasis (insulin, leptin and IGF-I). IGF-I and GH were conjointly assayed in the preprandial sample.

Thyroid hormones are critical for the maintenance of metabolic homeostasis and can markedly influence somatotrophic function. In a second experiment, thyroid function was thus evaluated in two groups of animals (scrapie affected vs healthy). The animals used for this experiment were the anoestrous ewes of objective 1. Blood samples were collected every hour for 24 h. Total thyroxine (T4) and tri-iodothyronine (T3) concentrations were determined in one every third sample and free thyroid hormone concentrations in every sample.

Assays

GH was measured in duplicate aliquots of plasma (100 μ l) with an RIA using a double-antibody separation method with reagents provided by the National Hormone and Pituitary Program (NHPP, Harbor UCLA R.E.I., St Torrance, CA, USA) and according to their recommended procedure. The results are expressed in terms of NHPP ovine GH-1–5. Two series of assays were conducted in two different locations with two different batches of reagents. For the first series, two assays were performed, sensitivity averaged 0.4 ng/ml for 100 μ l aliquots and the mean intra- and interassay coefficients of variation (C.V.) were 6.2 and 10.8% respectively. For the second series, two assays were performed, sensitivity averaged 3.0 ng/ml for 100 μ l aliquots and mean intra- and interassay C.V. values were 10.5 and 15.7% respectively.

Leptin was determined in duplicate on 100 μ l aliquots using a disequilibrium, double-antibody, ovine-specific RIA (Delavaud *et al.* 2000). Briefly, this assay utilised an anti-ovine leptin rabbit antibody at a final dilution of 1:30 000 and recombinant ovine [125 I]leptin, with recombinant ovine leptin as standard. Sensitivity was 0.8 ng/ml, and the intra- and interassay C.V. values were 6 and 9% respectively.

Total T3 and T4 were assayed in 50 and 20 μ l single plasma aliquots respectively, using a veterinary RIA kit from Beckman Coulter Immunotech (Marseilles, France). Assay sensitivity was 2.5 and 0.5 ng/ml for T4 and T3 respectively. Free T3 and T4 were assayed in 100 and

50 μ l single aliquots respectively, using Coat-A-Count RIA kits (Diagnostic Products Corp., Los Angeles, CA, USA), as previously validated for use in sheep (Moenter *et al.* 1991). The detection limit of the assay was 0.1 ng/ml and 0.5 pg/ml for T4 and T3 respectively.

Insulin was determined using the commercial INSULIN-CT RIA kit (CIS Bio International, Gif-sur-Yvette, France). The anti-insulin antiserum raised in guinea pig showed 100% cross-reactivity with bovine insulin, and the reliability of the assay for ovine insulin determination was verified by a parallelism test performed on serial dilutions of a pool of plasma from adult ewes. Plasma samples were analysed in duplicate according to the manufacturer's instructions and the intra- and inter-assay C.V. values were 8.8 and 11.7% respectively.

Metabolites were determined by enzymatic assays with an ELAN auto-analyser (Merck-Clevenot, Nogent sur Marne, France) (Ferlay & Chilliard 1999). Urea concentration was analysed with the Merck diagnostic kit (Chennevières-Lès-Louvres, France). NEFA concentration was measured with a Wako-Unipath NEFA-C kit (Oxoid, Dardilly, France). β -hydroxybutyrate concentrations and glucose concentrations were determined using the β -hydroxybutyrate dehydrogenase and glucose-dehydrogenase methods respectively. The intra- and inter-assay C.V. values were 0.5 and 7.0% for NEFA, 4.1 and 4.0% for β -hydroxybutyrate, 5 and 4% for urea and 0.5 and 2.0% for glucose respectively.

Plasma samples (25 μ l) were incubated in acid medium (0.01 M HCl) for 30 min at room temperature to dissociate IGFs from IGF-binding proteins (IGFBPs), then ultrafiltered on Centricon 30 (Amicon, Epernon, France) to separate IGFs from IGFBPs. The ultrafiltrate containing IGFs was lyophilised, then taken up in a solution containing 0.03 M NaH₂PO₄, 500 μ l/l Tween-20, 200 mg/l protamine sulfate, 200 mg/l NaNO₃ and 3.72 g/l EDTA (pH 7.4) and incubated for 2 days in a final volume of 500 μ l with a specific polyclonal anti-human IGF-I antibody (1:120 000 dilution) that cross-reacted with ovine IGF-I (gift from J Closset, Centre Hospitalo-Universitaire de Liege, Belgium) and 125 I-human IGF-I (10 000 c.p.m./tube). Iodination was performed using the iodogene method. Samples were tested at two concentrations plus one blank (without antibody), each in triplicate so as to confirm parallelism with the standard curve. After incubation, the free and bound IGFs were separated using albumin-coated charcoal. The threshold sensitivity of the assay was 1–2 ng/ml plasma. Intra- and interassay C.V. values were close to 5 and 10% respectively.

Data analysis

Pulses were identified using an adaptation of the method described by Wallace & MacNeilly (1986). Briefly, a pulse was identified as soon as the GH concentration reached a higher value than the mean value of the two preceding

points by at least two standard deviations. The pulse that lasted as long as the GH concentration at a given time remained higher than the mean value of the two following points by at least two standard deviations. The standard deviation under consideration was estimated for each point from the mean intra-assay C.V. according to the following equation: $S.D. = C.V. \times \text{mean concentration} / 100$.

When variances between groups were non-homogenous, the data were log transformed before statistical analysis. GH concentration profiles over time were compared between healthy and scrapie-affected ewes using repeated measures ANOVA including ewes as a random effect factor, and time, group and their interactions as fixed effect factors. The effect of the adrenergic agonist on GH concentrations was analysed using a linear model including ewes as a random effect factor, and period relative to the agonist administration, time within period and their interactions as fixed effect factors. The area under the curve (AUC) ratios, mean GH concentrations, pulse amplitude, mean GH concentration during interpulses, GH mean concentration and age were compared between healthy and scrapie-affected ewes using an unpaired *t*-test. Pulse frequencies were analysed with a non-parametric Mann–Whitney test. Metabolic parameters were analysed using a repeated measures ANOVA, including ewes as a random effect factor, and meal, group and their interactions as fixed effect factors. In objective 4, the relative effects of disease and GH on IGF-I concentration were analysed using a linear model including the disease as a fixed effect factor and GH concentration as a covariate.

Results

Objective 1: Characterisation of scrapie-associated alterations of somatotrophic function: modification of mean GH concentration

The GH secretory profiles in hourly samples differed between scrapie-affected and healthy ewes (interaction daytime $\times$ group $P < 0.05$). This difference was observed both in animals sampled during the breeding season and in animals sampled during the anoestrous season. This resulted in higher mean GH concentrations in scrapie-affected ewes (Fig. 1). Similarly, the GH concentrations in males were more than 2-fold higher in scrapie-affected animals (means $\pm$ S.E.M.: 4.8 ± 1.5 ng/ml vs 1.9 ± 0.4 ng/ml for healthy rams; $P < 0.05$).

In our epidemiological survey, the mean GH concentration in scrapie-affected sheep was higher than in healthy controls (means $\pm$ S.E.M.: 4.0 ± 0.7 and 1.4 ± 0.3 ng/ml for scrapie-affected and healthy ewes respectively; $P < 0.001$). In contrast, mean ages did not differ significantly between the two populations (3.2 ± 0.3 and 2.5 ± 0.3 years for healthy and scrapie-affected ewes respectively). GH concentrations measured in a single sample showed high inter-individual variability which was probably due to the

Figure 1 Mean $\pm$ S.E.M. GH concentrations in scrapie-affected (solid bars) and healthy (open bars) ovary-intact ewes during the breeding (top panel) or anoestrous (bottom panel) seasons. GH was measured in samples collected every hour for 10 h from one set of animals during the breeding season and for 24 h from another set of animals during the anoestrous season. * $P < 0.05$.

pulsatile secretion of this hormone. It is noteworthy, however, that despite this variability, the large number of animals included in the survey enabled us to show a statistically significant difference in GH concentration between healthy and scrapie-affected ewes.

Objective 2: Are the effects of scrapie on mean GH concentration related to alteration of pulsatility?

The mean GH concentration over 24 h and the mean concentrations of interpulse episodes tended to be higher in scrapie-affected ewes ($P = 0.07$ and 0.08 respectively). Figure 2 depicts representative examples of GH secretory profiles from one healthy control and one scrapie-affected ewe and GH mean $\pm$ S.E.M. concentrations in both groups. The GH pulse frequency was significantly higher in scrapie-affected ewes ($P = 0.04$), but neither pulse amplitude nor mean pulse area differed between the groups (Fig. 3; $P = 0.5$ and 0.4 for amplitude and area respectively).

Objective 3: Are the effects of scrapie on mean GH concentration related to responsiveness to α_2 -adrenergic stimulation?

Mean GH concentrations during the control period were significantly higher in scrapie-affected ewes ($P < 0.05$). As a result, the AUC of GH concentration vs time during the control period was 3.3-fold higher in scrapie-affected than in healthy ewes (means $\pm$ S.E.M.: 33.5 ± 10.5 and

Figure 2 Representative examples of GH secretory profiles in one scrapie-affected (top panel) and one control healthy (bottom panel) ewe. GH was measured in blood samples collected every 30 min for 24 h during the anoestrous season in ovary-intact ewes. Arrows indicate identified pulses. The bar graph depicts the mean $\pm$ S.E.M. GH concentrations in both groups. $P=0.07$.

10.1 ± 2.6 ng.h/ml for scrapie-affected and control ewes respectively). Romifidin administration induced a dramatic increase in GH concentrations within 15 min in both groups. The GH AUC following the administration of 0.05 mg/kg Romifidin was 2.4-fold higher in scrapie-affected than in healthy ewes (means $\pm$ S.E.M.: 98.1 ± 37.2 and 41.2 ± 9.7 ng.h/ml for scrapie-affected and control ewes respectively). However, the ratio of the AUC post-injection to the AUC pre-injection did not differ between groups (Fig. 4).

Objective 4: Is scrapie associated with metabolic disturbances?

At the time of this experiment, body weights were not different between healthy and scrapie-affected ewes (mean $\pm$ S.E.M.: body weight 40 ± 2 and 37 ± 2 kg for healthy and scrapie-affected ewes respectively; $P=0.3$) suggesting that body conditions were similar between groups. In agreement with this observation, no difference in mean leptin concentration could be seen. The recording of the concentrate intake (the main source of energy) did not show any difference between the two groups. Concentrate intake had no significant effect on the hormonal or metabolic parameters measured except for NEFA (Table 1). Indeed, a significant interaction between group and period (before or after the meal) was observed,

characterised by a decrease in circulating concentrations of NEFA 4 h after the concentrate meal in scrapie-affected ewes only. Independently of food intake, scrapie-affected ewes showed higher plasma glucose concentrations (Table 1) associated with higher insulin ($P<0.05$) and urea ($P<0.05$) concentrations. IGF-I concentration did not differ significantly between groups ($P>0.05$). No significant interactions between group and GH concentration measured in the preprandial sample on IGF-I concentration could be observed ($P=0.8$).

In the second experiment, thyroid status was assessed in two other groups of animals (healthy and scrapie affected). Mean total T4 concentration was significantly lower in scrapie-affected ewes (means $\pm$ S.E.M.: 75.8 ± 5.1 and 58.0 ± 2.7 ng/ml in healthy and scrapie-affected ewes respectively). In contrast, neither total T3, nor free T3 and T4 concentrations differed significantly between groups (means $\pm$ S.E.M. for total T3: 1.5 ± 0.1 and 1.3 ± 0.09 ng/ml; free T4: 0.95 ± 0.06 and 0.75 ± 0.08 ng/ml; free T3: 1.73 ± 0.09 and 1.42 ± 0.22 pg/ml for healthy and scrapie-affected groups respectively).

Discussion

Our study has clearly demonstrated that scrapie is associated with alterations in GH secretion. These alterations are

Figure 3 Mean $\pm$ S.E.M. number of GH pulses/24 h, AUC and amplitude of GH pulses and GH concentrations during interpulse episodes in scrapie-affected (solid bars) and healthy control (open bars) ovary-intact ewes. $P=0.04$, 0.4 , 0.5 and 0.08 for pulse number, pulse AUC, amplitude and interpulse GH concentration respectively.

Figure 4 The left panel indicates the mean $\pm$ S.E.M. GH concentrations in blood samples collected every 15 min for 3 h before and 3 h after an i.v. injection of an α_2 -adrenergic agonist (Romifidin, 0.05 mg/kg at 0 min) in scrapie-affected ($n=4$) and healthy control ($n=4$) ovary-intact ewes. The left panel describes the mean $\pm$ S.E.M. ratio of AUC of GH vs time after to the GH AUC before agonist administration in both groups.

Table 1 Effect of scrapie and meal on the metabolic status of adult ovary-intact ewes. Values are expressed as means $\pm$ S.E.M. These parameters were measured in blood samples collected 4 h before (preprandial) and 4 h after (postprandial) a concentrate meal

	Scrapie		Control	
	Preprandial	Postprandial	Preprandial	Postprandial
IGF-I (ng/ml)	133 $\pm$ 23		102 $\pm$ 21	
GH (ng/ml)	11.6 $\pm$ 2.8		8.0 $\pm$ 3.3	
Leptin (ng/ml)	3.7 $\pm$ 0.1	3.6 $\pm$ 0.1	4.4 $\pm$ 0.5	4.4 $\pm$ 0.4
Insulin (μ U/ml)*	24 $\pm$ 5	28 $\pm$ 7	11 $\pm$ 1	15 $\pm$ 3
Glucose (g/l)*	0.74 $\pm$ 0.04	0.74 $\pm$ 0.03	0.58 $\pm$ 0.03	0.57 $\pm$ 0.03
NEFA (mmol/l)†	0.36 $\pm$ 0.07	0.13 $\pm$ 0.03	0.29 $\pm$ 0.08	0.30 $\pm$ 0.11
3-OH-butyrate (mmol/l)	0.34 $\pm$ 0.04	0.41 $\pm$ 0.03	0.42 $\pm$ 0.10	0.43 $\pm$ 0.07
Urea (g/l)*	0.32 $\pm$ 0.02	0.33 $\pm$ 0.02	0.23 $\pm$ 0.05	0.24 $\pm$ 0.04

* $P < 0.05$, effect of the disease; † $P < 0.05$, interaction between meal and disease.

characterised by an increase in GH plasma concentrations observed during both the breeding and the anoestrous seasons and in both sexes. According to our epidemiological survey, age is unlikely to be a determining factor for the difference in GH concentration observed between healthy and scrapie-affected sheep. Higher GH concentration results, at least in part, from increased pulse frequency suggesting that central mechanisms are involved. However, it is unlikely that an increased responsiveness to α_2 -adrenergic central stimulatory pathways is involved. Finally, multiple metabolic disorders (increased glycaemia, insulinaemia, uraemia and higher responsiveness of NEFA to concentrate meal intake) were observed in scrapie-affected animals.

Although scrapie can be considered as a long-lasting chronic disease, our clinical data do not fit the classical pathophysiological endocrine scheme of such conditions. Indeed, chronic diseases such as arthritis (Templ *et al.* 1996, Lopez-Calderon *et al.* 1999), parasitism (Elsasser *et al.* 1988) and the chronic phase of critical illness (Ligtenberg *et al.* 2001, Van Den Berghe 2002) are all associated with a decrease in one or more pituitary functions, including somatotrophic functions, which results in a catabolic and wasting syndrome state.

In contrast, a stimulation of GH secretion is often observed during the initial phase of acute illness and in experimental models of acute diseases such as endotoxin challenge in sheep (Briard *et al.* 1998) and humans (Lang *et al.* 1997). Increased exposure to GH, in acute pathologies associated with severe catabolism and malnutrition, is believed to allow full expression of beneficial effects related to the initial endocrine stress response. For example, the stimulation of GH secretion in acute illness results in direct lipolytic, insulin-antagonising and immune stimulatory actions (Van Den Berghe 2002). Although scrapie is a long-lasting chronic wasting disease, most of our observations seem to fit the pathophysiological endocrine scheme of acute illness. In particular, our results clearly suggest that a relative state of insulinoreistance,

characterised by increased glycaemia despite an increase in insulinaemia, might occur in scrapie-affected sheep. Increased GH concentration in scrapie-affected ewes is likely to mediate a state of insulinoreistance (Clemmons 2004). Alternatively, this state may be related to prion-induced alterations of insulin receptor gene expression and functionality as shown in prion-infected neuroblastoma cells (Östlund *et al.* 2001b).

Non-metabolic beneficial effects might arise from increased GH secretion. Indeed, GH exhibits IGF-I-independent neuroprotective effects (Scheepens *et al.* 2001) and can induce the expression of suppressors of cytokine signalling (Adams *et al.* 1998, Davey *et al.* 1999, Wu *et al.* 2003) particularly during the acute phase of different injuries. Scrapie, like other natural or experimental spongiform encephalopathies, is associated with an increased expression of cytokines in the central nervous system, in particular tumour necrosis factor- α and interleukin-1 (Williams *et al.* 1994, 1997, Kim *et al.* 1999, Baker *et al.* 2002, Eikelenbroom *et al.* 2002). Increased GH concentration might therefore help to contain the devastating effects of prolonged exposure of the brain to cytokines and be part of the panel of mechanisms aimed at limiting the neurodegenerative process.

Putative mechanisms underlying the stimulation of GH secretion remain to be determined. In particular, the question arises as to whether central mechanisms are involved. Pulsatility of GH secretion typically results from central mechanisms, namely hypothalamic inhibitory somatostatinergic and stimulatory GH-releasing hormone (GHRH) tones (Robinson 1991). Our results show that GH pulsatile secretion is modified in scrapie-affected ewes, which therefore suggests that central mechanisms are involved in the stimulation of GH secretion observed in such animals. GHRH and somatostatin secretions are themselves under the control of several neuromediators. For example, the stimulation of GH secretion by α_2 -adrenergic agonists in sheep results from an increase in GHRH secretion in the portal blood (Magnan *et al.* 1994).

Interestingly, our observation that GH response to an α_2 -adrenergic agonist is not modified by scrapie suggests that the responsiveness of the central component of the somatotrophic axis to α_2 -adrenergic stimulatory pathways is not profoundly modified by scrapie. Cytokines can also alter somatotrophic functions at a central level. It is therefore tempting to hypothesise that scrapie-related cytokine up-regulation in the diseased brains may mediate the stimulation of somatotrophic function.

Alterations in GH secretion could result from intimate dysfunctioning of the main regulatory mechanisms of the hypothalamo-hypophysial somatotrophic axis and, in particular, at the level of the interactions between GH and IGF-I. For example, in many pathological situations associated with severe catabolism, the regulation of IGF-I and GH secretions appears to be uncoupled through a mechanism of acquired resistance to GH. IGF-I secretion thus escapes GH stimulatory control, and this results in a decrease in IGF-I concentration leading to a low level of negative feedback of IGF-I on GH secretion (Van Den Berghe 2002). Our results failed to demonstrate a reduction in total IGF-I concentration typical of GH-acquired resistance in scrapie-affected ewes. However, the fact that IGF-I was not increased in those ewes having shown increased GH pulse frequency suggests that a certain degree of dissociation between GH and IGF-I secretion might occur in scrapie-affected ewes. Thus, in the absence of direct assessment of IGF-I responsiveness to the stimulatory action of GH, the hypothesis of a scrapie-associated state of acquired GH resistance cannot be completely ruled out. Another possible and clinically relevant endocrine interaction that might explain our results would be a decrease in the efficacy of the IGF-I negative feedback. Such a reduction in the negative feedback of IGF-I on GH secretion could reflect a general lack of action of IGF-I. This mechanistic hypothesis is supported by *in vitro* data obtained from scrapie-infected neuroblastoma cells which showed an over-expression of a truncated functionally impaired IGF-I receptor (Satoh *et al.* 2000). Further investigations are required to demonstrate a lack of action of IGF-I *in vivo* in transmissible spongiform encephalopathies.

Metabolic homeostasis is intimately linked to the somatotrophic function as well as other endocrine systems. Thyroid hormones and leptin are two endocrine systems critical for metabolic homeostasis that can interact with somatotrophic function. The present study failed to show alterations of leptin concentration in scrapie-affected ewes. Modifications of the thyroid axis were limited to a decrease in total T4 but no modification of total T3 and free T3 and T4 concentrations. Thus, it was difficult to relate GH modification to alterations in thyroid functions. It is interesting to note, however, that this decrease in total T4 concentration could be related to a decrease in thyrotrophin secretion.

Our results have shown the existence of endocrine alterations typical of acute illness in scrapie-affected sheep.

It is tempting to hypothesise that the maintenance of those endocrine alterations in this chronic disease is related to a high level of expression of cytokines in the diseased brain. Such a hypothesis assumes that the central components of the somatotrophic axis, namely GHRH and somatostatin secretions, are altered in scrapie. The modifications in GH pulse frequency observed in this study are consistent with such a hypothesis.

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