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Prominent corticosteroid disturbance in experimental prion disease

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Abstract

Prion diseases comprise a group of neurodegenerative disorders that invariably lead to death in affected individuals. The most prominent event in these diseases is a rapid and pronounced neuronal loss, although the cause and the precise mechanisms of neuronal cell death have not been identified so far. Recently, it has been suggested that corticosteroids might play a role in the pathogenesis of neurodegenerative disorders in general, as the regulation of these hormones was found to be disturbed in Alzheimer's and Parkinson's disease, as well as in a transgenic mouse model of Alzheimer's disease. To evaluate the possible corticosteroid disturbances in prion diseases, we determined the concentration of corticosterone metabolites in the faeces of scrapie-inoculated mice during the course of the clinical disease. We observed markedly elevated concentrations of the metabolites during the last 5 weeks of the disease, as well as a severe disturbance of circadian periodicity of corticosterone excretion as much as 2 weeks before this elevation. A simultaneous downregulation of cerebral neuronal glucocorticoid receptors was not detectable by immunohistochemistry, indicating that increased corticosteroids can elicit their effects in mouse scrapie freely. The dysregulation of corticosteroid excretion might act as a further cofactor in the pathogenesis of scrapie, for example by preconditioning nerve cells to disease-immanent neurotoxic stimuli, such as oxidative stress, and to apoptosis.

Introduction

Prion diseases are fatal neurodegenerative disorders characterized by an accumulation of a pathological, partially protease-resistant isoform (PrP^{Sc}) of the normal cellular prion protein (PrP^{C}) mainly in the brain of affected individuals, and by pronounced neuronal loss. The precise mechanisms of neuronal death have not been clarified so far (Unterberger *et al*., 2005). Many analogies between prion diseases and other neurodegenerative disorders have been demonstrated, for example an involvement of oxidative stress, apoptotic pathways and complement activation (Gray *et al*., 1999; Guentchev *et al*., 2002; Kovacs *et al*., 2004). Recently, it was suggested that corticosteroids might play a role in the pathogenesis of neurodegenerative disorders in general, as the regulation of these hormones was found to be disturbed in Alzheimer's and Parkinson's disease in humans, and in a mouse model of Alzheimer's disease (Davis *et al*., 1986; Maeda *et al*., 1991; Hartmann *et al*., 1997; Peskind *et al*., 2001; Touma *et al*., 2004a). Interestingly, some clinical features in prion diseases, such as behavioural disturbances, stimulus-sensitive myoclonus or startle reactions and sleeping disturbances in humans (WHO, 2003b), as well as restlessness, hyperexcitability, stimulus-sensitive cloni or startle reactions and aggressiveness in animals (WHO, 2003a; personal observations), point towards a stress reaction of the organism. It is therefore important to analyse the stress system in prion diseases.

The two major components of the stress system are the sympathetic-adrenomedullary system and the hypothalamic-pituitary-adrenocortical (HPA) axis (Axelrod & Reisine, 1984; Bornstein & Chrousos, 1999). The sympathetic-adrenomedullary system responds mainly to acute stressors whereas the HPA axis is involved in the reaction to acute, subacute and chronic stress events (Keller-Wood & Dallman, 1984). For chronic disorders such as prion diseases, the HPA axis is the system of choice to determine the presence and the extent of a stress reaction. In fatal familial insomnia (FFI), a human prion disease with prominent thalamic pathology (Lugaresi *et al*., 1987; Portaluppi *et al*., 1994), and sheep scrapie (Schelcher *et al*., 1999; Gayrard *et al*., 2000; Picard-Hagen *et al*., 2000), elevated plasma cortisol concentrations are seen. In FFI, the circadian rhythm of cortisol and corticotropin (ACTH) secretion is disturbed (Portaluppi *et al*., 1994); however, this could not be verified in scrapie, as the respective study failed to detect any circadian secretion rhythm in sheep in general (Gayrard *et al*., 2000), probably because of the invasive sampling procedure used. Meanwhile, assays for corticosteroid metabolites in faeces were developed, by which adrenocortical activity can be determined non-invasively (Möstl & Palme, 2002; Touma *et al*., 2003, 2004b). Such non-invasive assaying reduces the exogenous disturbance of the animal under investigation to a minimum, leaving its natural circadian rhythm almost undisturbed, a fact particularly important for sequential measurements that are necessary to study the diurnal variation of glucocorticoids. In addition,

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serial blood sampling in mice is limited by the small volume of blood in healthy, and even more so in scrapie-affected, animals. Finally, determination of the concentrations of hormone metabolites in pooled faeces rules out any influence of episodic plasma peaks. Also noteworthy is that metabolite concentrations in faeces reflect the active corticosteroid fraction in plasma, as only free steroids are eliminated from the plasma compartment.

To characterize adrenocortical activity, and to evaluate any disturbance of circadian periodicity in mouse scrapie, we determined corticosterone (which is the main corticosteroid in mice) metabolite concentrations in 24-h pooled faecal samples, and also their variation within complete circadian cycles weekly in scrapie-inoculated mice during the whole clinical phase of the disease. Furthermore, we analysed the expression of glucocorticoid receptors (GR) in the brains of mice with end-stage disease in order to assess whether corticosteroids can actually elicit their effects in the diseased brain.

Materials and methods

Animals and housing conditions

A total of 32 adult female C57Bl/6 J mice (obtained from Institute for breeding and genetics of laboratory animals, department for biomedical research, Medical University of Vienna, A-2325 Himberg, Austria) were used in this experiment, 20 being used for the assessment of faecal corticosterone metabolites and 12 being used for the immunohistochemical characterization of GR expression in the brain. At 10 weeks of age, 16 mice were inoculated intraperitoneally with 100 μL Rocky Mountain Laboratory strain of scrapie (RML; courtesy of M. Groschup, Riems, Germany) at a 1 : 100 dilution. All mice were maintained under standard laboratory conditions (i.e. an artificial day-night cycle with light from 06.00 h to 18.00 h, and constant temperature/humidity). To make the animals less sensitive to exogenous disturbances such as subdued conversation, a radio was kept on at low volume day and night. Primarily, scrapie-inoculated, as well as control mice, were kept in groups of five to six animals in type III standard polycarbonate cages (38 × 22 × 15 cm). Three weeks before the beginning of sample collection, each mouse was placed in a separate cage of the same size. All animals had free access to food pellets and water. To minimize discomfort, clinically disabled scrapie-inoculated mice received additional water-soaked pellets at least twice a day. Scrapie-inoculated mice were killed at the final stage of the disease, when they refused further food intake. All controls were killed together 10 days after the last animal with scrapie. The experimental setup was approved by the local animal welfare committee of the General Hospital, Vienna, Austria.

Cage preparation

Mice with scrapie become increasingly disabled during the development of the clinical disease. Therefore, the use of conventional metabolic cages was not feasible for practical, as well as ethical, reasons. To allow fast and accurate sampling of faeces, the conventional polycarbonate cages were adapted as follows: a layer of sawdust was covered with three linen sheets that were fixed around the edge with plastic frames; an additional crumpled sheet was placed into the cage to be used by the animal as a shelter. The sawdust and sheets were changed weekly 24 h before the next sampling session.

Sample collection

Sample collection started when four scrapie-inoculated animals showed the first discrete neurological symptoms (incipient gait disturbance, and impairment in the 'vertical pole' and 'hanging wire' tests as described in Crawley, 2000), and three animals had developed unequivocal disease symptoms, while the remaining three inoculated mice were still clinically healthy. Sampling was performed weekly according to the following schedule: for the first 24 h after cage preparation (i.e. from 11.00 h to 11.00 h the following day), the animals were allowed to habituate to their new surroundings. All faeces voided within this period were discarded. Faeces produced during the next 24 h were collected for the determination of the mean corticosterone metabolite concentration within one circadian cycle (24 h samples). To determine the diurnal variation of corticosterone metabolites during the subsequent 24 h, sampling was carried out at intervals of 2 or 4 h, respectively, at 15.00 h, 17.00 h, 19.00 h, 21.00 h and 23.00 h, and 01.00 h, 03.00 h, 07.00 h and 11.00 h. To minimize external stressors, the animals were not touched directly during sampling, and sample collections during the dark phase of the day-night cycle were performed under dimmed light conditions. The faecal samples were frozen within 60 min of collection and kept at −20 °C until required for the analyses.

Analysis of faecal corticosterone metabolite concentrations

The faecal steroid metabolites were extracted according to the method described by Touma *et al*. (2003). Briefly, each sample was homogenized, and an aliquot of 0.05 g was extracted with 1 mL 80% methanol. After centrifugation at 2500 ***g*** for 10 min, an aliquot of the supernatant was diluted 1 : 10 with assay buffer (Tris/HCl 20 mM, pH 7.5), and analysed with a 5α-pregnane-3β,11β,21-triol-20-one enzyme immunoassay (EIA). In principle, this EIA is based on a double-antibody technique using a biotinylated label as a competitor for corticosterone metabolites (for details regarding analytical procedure, biochemical characteristics and biological validation of this assay see Touma *et al*., 2003 and 2004b). The intra- and interassay variation coefficients were 9.1% and 14.0%, respectively.

Histology and immunohistochemistry

Animals were killed with CO_2 and immediately perfused transcardially with ice-cold phosphate-buffered saline (PBS), followed by 4% buffered formalin. Subsequently, whole skulls including the brain were fixed in formalin for 48 h. After rinsing in PBS for 3–5 days, the skulls were decalcified by immersion in 0.5 M EDTA (pH 8.0) for at least 2 weeks. Coronary slices at the level of the frontal cortex and caudoputamen, and, for the analysis of GRs, at the level of the anterior portion of the hippocampus, were embedded in paraffin. Haematoxylin-eosin-stained specimens (4 μm thick) were prepared for histological assessment. A monoclonal antibody against PrP (clone 6H4; Prionics, Schlieren, Switzerland) was used at a dilution of 1 : 500 for the immunohistochemical confirmation of the diagnosis of scrapie. The sections for PrP^{Sc} labelling were pretreated as described previously (Voigtländer *et al*., 2001). Briefly, a three-step protocol, including hydrated autoclaving, incubation in concentrated formic acid and, subsequently, incubation in guanidine isothiocyanate, was used. Pretreatment of the sections for immunohistochemical detection of GR included steaming for 1 h in citric acid buffer (pH 6.0). The GR antibodies (mouse monoclonal clone 3D5, and rabbit polyclonal; both obtained from Novus Biologicals, Littleton, CO, USA) were diluted 1 : 500 in a detergent-containing antibody diluent (DAKO, Glostrup, Denmark) and left on the slides overnight at 4 °C. For all immunohistochemical stainings, a labelled

streptavidin biotin (LSAB) + peroxidase detection kit (DAKO) was used for the visualization of the antibody reaction. The specificity of primary antibodies was verified by using appropriate serum and isotype controls.

Neuronal expression of GRs was analysed in the hippocampus (subfields CA1, CA2, CA3, CA4 and dentate gyrus), the caudoputamen and the retrosplenial cortex in a blinded manner. The signal intensity of GR-immunoreactive neuronal nuclei was assessed semiquantitatively according to the following schema: +++, strong nuclear labelling; ++, moderate labelling; +, weak labelling; and 0, no detectable labelling. In the hippocampus, the relative distribution of stained and unstained neuronal nuclei was assessed by calculating the number of immunopositive (+++, ++, +) and immunonegative nuclei in a representative area of each subfield.

Statistical analysis

Statistical data analysis was carried out using the SPSS 10.0 program package (SPSS Inc., Chicago, IL, USA). The concentrations of the faecal corticosterone metabolites of control mice were distributed normally; as this was usually not the case for scrapie-inoculated mice, the data are given as medians and ranges. Scrapie-inoculated mice reached the endpoint of the study (i.e. the ultimate stage of clinical disease, shortly before spontaneous death was expected to occur) with considerable variations. To render the collective statistical evaluation of the corticosterone metabolite levels in parallel with the disease course possible, data for scrapie-inoculated animals were adjusted as follows: week 0 is the week in which the last samples were collected before the respective animal had to be killed as a result of disease progression. Accordingly, weeks -1 to −10 correspond to the 10 previous sampling sessions. The significance of differences between data points was evaluated by the Mann–Whitney U (MWU)-test (two-tailed).

Results

Incubation time, duration of disease and disease phenotype

All scrapie-inoculated animals developed typical clinical signs within 177.6 ± 19.9 days (mean ± SD) after inoculation, and were killed at the ultimate stage of the disease at an average age of 221.8 ± 17.8 days (mean ± SD). The duration of overt clinical disease in individual mice ranged from 5 to 9 weeks. Disease onset in scrapie-infected mice was characterized by a slight impairment of motor coordination (in the 'hanging wire' and 'vertical pool' tests, according to Crawley, 2000). Soon, further neurological, as well as behavioural, symptoms developed, such as restlessness, vertical leaping, gait disturbance and, finally, overt ataxia. In this early-to-mid phase, stimulus sensitive cloni and/or startle reactions could also be evoked. Towards the final disease stage, some animals presented with signs of a spastic paraplegia, whereas others showed a sustained irregular motor hyperactivity, interrupted by unresponsive and akinetic intervals. The development of neurological symptoms was paralleled by a slight disturbance, and later a complete loss, of the animal's natural resting-activity cycle. Finally, within their last 4-5 weeks, scrapie-infected animals lost approximately one-third of their maximal body weight. In contrast, none of the control animals showed any neurological or other symptoms throughout the whole study. The diagnosis of scrapie was confirmed both histologically and immunohistochemically. The brains of control animals displayed neither histological features of scrapie, such as spongiform change, gliosis and neuronal loss, nor deposition of PrP^{Sc} (data not shown).

Concentration of faecal corticosterone metabolites in 24-h pool samples

A summary of faecal corticosterone metabolite concentrations in 24-h pool samples is presented in Fig. 1. For collective and statistical analyses, data were adjusted as described in the methods section. Throughout the whole study, faecal corticosterone metabolite concentrations did not change significantly in control mice (MWU test, $U = 31$–49; $P > 0.05$ for all sampling weeks). In contrast, scrapie-inoculated mice showed elevated levels of faecal metabolites from week −4 to week 0 (MWU test, $U = 7$–10, $P < 0.01$ for weeks −4 and −3; $U = 0$–3, $P < 0.001$ for weeks −2 to 0; Fig. 1). In addition, when investigating fecal corticosterone metabolite concentrations within the group of scrapie-inoculated animals, values rose significantly in weeks −1 and 0 as compared with the respective previous week (MWU test, $U = 22$, $P < 0.05$ in week −1; $U = 12$, $P < 0.01$ in week 0; Fig. 1). In week 0, control animals had a median corticosterone metabolite concentration of 176 ng/50 mg faeces (range 104–271 ng/50 mg), whereas scrapie-inoculated animals displayed a manifold (on average 4.3-fold) increase in metabolite concentration, having a median value of 749 ng/50 mg faeces (range 543–1316 ng/50 mg). It should be emphasized that the trend of the curve was not quite the same in all scrapie animals. Whereas some animals had metabolite concentrations that were within the overall range of controls (80–411 ng/50 mg faeces) until week −1, and showed an increase only immediately before their deaths, the majority already had elevated levels 1–6 weeks earlier, and then displayed a final increase in week 0.

Circadian pattern of faecal corticosterone metabolite concentrations

Again, for statistical analysis, data were arranged as described in the methods section. For the 11 weeks in which the diurnal variation of faecal corticosterone metabolite concentrations was determined, sampling weeks 0, −2, −4, −6, and −8 are shown as representatives in Fig. 2. In control mice, a regular circadian rhythm was evident throughout the whole study, with the highest metabolite concentrations usually observed between 21.00 h and 01.00 h. After this plateau, levels began to sink and were lowest from 11.00 h to 17.00 h (Fig. 2). This course of diurnal corticosterone metabolite concentration corresponds to that observed in previous studies (Touma *et al*., 2004b), and reflects the plasma concentration of corticosterone with a delay of 6–8 h resulting from the time needed for metabolization, biliary secretion and transport through the gut.

From week −10 to week −8, there was no difference between controls and scrapie-inoculated mice with respect to circadian variation and concentration of excreted corticosterone metabolites (Fig. 2; see also Supplementary material, Fig S1). In weeks −7 and −6, an elevation in scrapie-infected animals occurred, which was statistically significant at some time points in week −7, and at all except two time points in week −6 (MWU test $U = 3$–18, $P < 0.05$ at 19.00 h, 01.00 h and 03.00 h in week −7; $U = 2$–19, $P < 0.05$ and $P < 0.01$ in week −6 except at 03.00 h and 11.00 h; Fig. 2). However, the circadian rhythm was still preserved. From week −5 onwards, the diurnal excretion pattern seemed disturbed, as no regular nightly increase in faecal corticosterone metabolite concentrations could be observed further, and levels were often higher by day than by night. From week −4, all values (with one exception in week −3) at all time points in scrapie-infected mice were increased significantly as compared with controls, and the disturbance of the circadian pattern became even more pronounced until week 0 (MWU test $U = 0$–21.5,

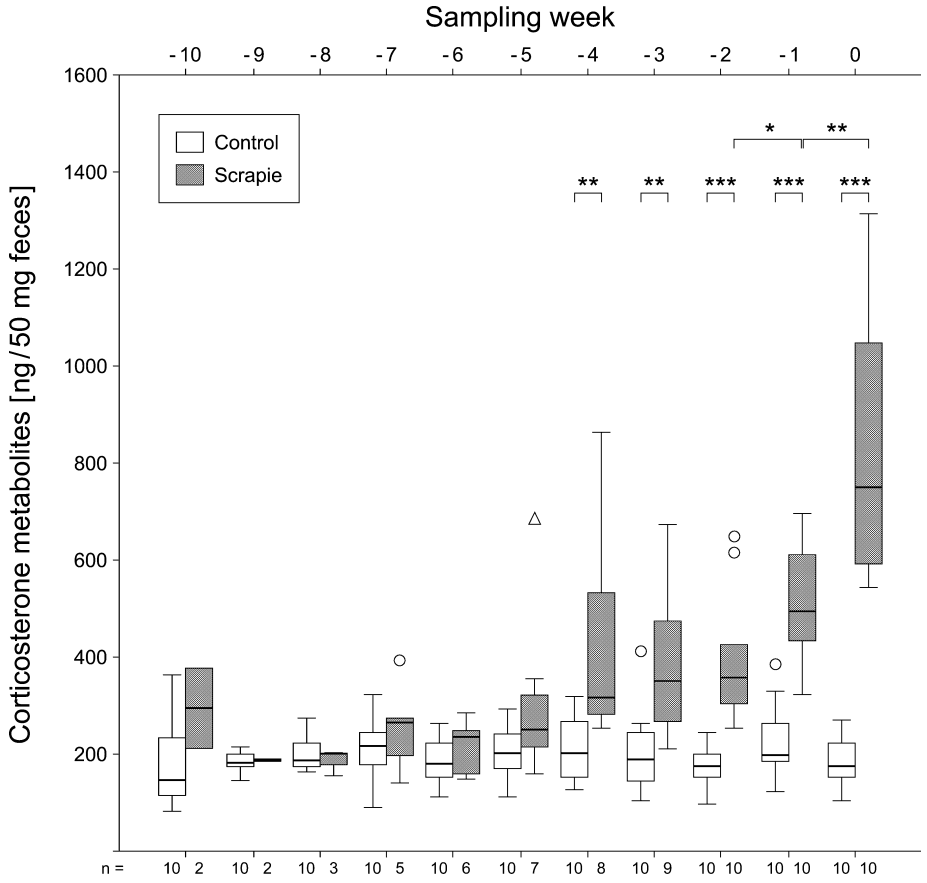

FIG. 1. Time course of faecal corticosterone metabolite concentration in 24-h pool samples of scrapie-infected and of control mice. Week 0 is the week in which the last samples were collected before the respective animal was killed. Accordingly, weeks −1 to −10 correspond to the 10 previous sampling sessions. The number of mice included in the calculation at the respective time point is given on the horizontal axis. Open circles in the box-whisker plot represent outliers; open triangles represent extreme outliers. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

$P < 0.05$ to $P < 0.001$; Fig. 2). In addition, the ranges of corticosterone metabolite concentrations within the scrapie group widened markedly towards the end of the disease, reflecting the increasingly irregular profiles of individual animals.

Expression of cerebral GRs

Expression of neuronal GRs was assessed by means of immunohistochemistry. In all regions investigated, staining of GRs was localized to the nucleus, a characteristic feature of steroid receptors in the presence of a sufficient amount of ligand. As a principal staining pattern, we observed a moderate-to-strong labelling of nuclei in the hippocampal subfields CA1 and CA2, as well as in the retrosplenial frontal cortex, a moderate labelling in the dentate gyrus of the hippocampus, and a weak-to-moderate labelling in the hippocampal subfields CA3 and CA4 (Fig. 3A and B). Apart from the moderate staining in CA3 and CA4, this distribution is in agreement with previous reports on GR-immunoreactive cells in the rat central nervous system (for review see Sanchez *et al*., 2000). In general, there was a slight variation of the nuclear staining intensity between individual animals of both the scrapie and the control group. Despite this variation, semiquantitative analysis of hippocampal and neocortical neuronal GR immunoreactivity revealed no overt differential receptor regulation in scrapie-inoculated mice as compared with control animals (Fig. 3A and B).

Discussion

We demonstrate here for the first time: (i) a highly significant overall elevation of faecal corticosterone metabolite concentrations; (ii) a general disturbance and, in the final stage, a loss of circadian periodicity of corticosterone metabolite excretion; and (iii), no overt downregulation of neuronal GRs in all analysed brain regions in scrapie-affected mice. These results are indicative of a severe, chronic overactivation of the HPA axis in murine scrapie, and of a disturbance of circadian regulation in general. Importantly, corticosterone can elicit its effects freely in the diseased brain, as there is no substantial change in the expression of neuronal GRs.

In the physiological situation, the response of the HPA axis to an acute stressor is limited by negative feedback mechanisms acting at different sites of the brain, including the hippocampus (the brain region with the highest content of both mineralocorticoid and glucocorticoid receptors in the majority of species assessed) and the hypothalamus (Jacobson & Sapolsky, 1991; Höschl & Hajek, 2001; Lathe, 2001). During chronic stress, inhibitory feedback regulation is diminished or even completely absent. Plasma levels of corticotropin releasing hormone (CRH) and corticotropin (ACTH) usually remain in the normal range despite increased corticosteroid concentrations, resulting in a continuous stimulation of the adrenal cortex. To make matters worse, the adrenal cortex reacts to sustained stimulation with hypertrophy, cell hyperplasia and, subsequently, with enhanced sensitivity to ACTH.

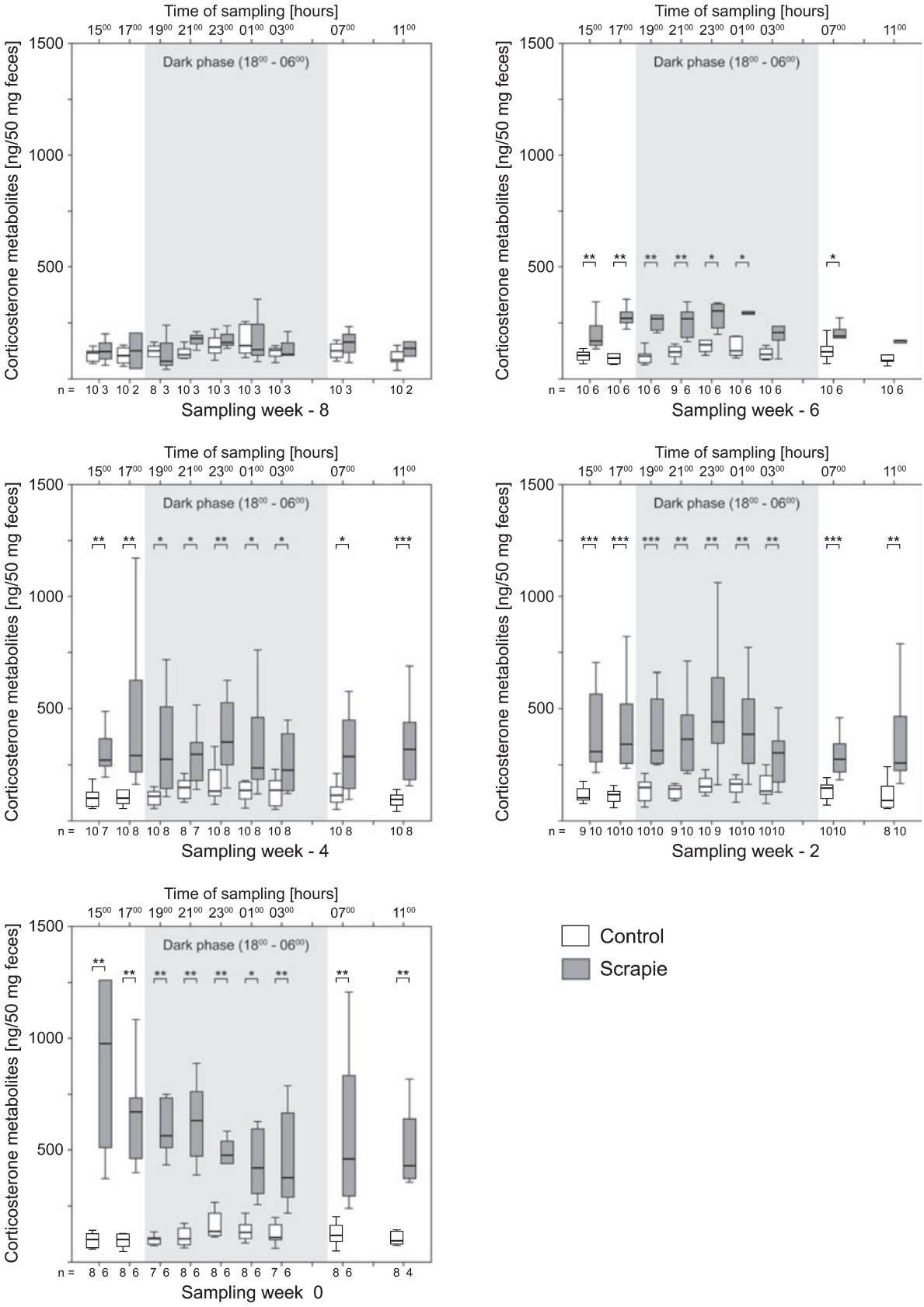

FIG. 2. Circadian pattern of the corticosterone metabolite concentration of scrapie-infected and control mice during the course of the disease. Week 0 represents the week in which the last samples were collected before the respective animal was killed. Weeks −2, −4, −6 and −8 correspond to four of the previous sampling sessions. The number of mice included in the calculation at the respective time point is given on the horizontal axis. To improve the clarity of the presentation, outliers and extreme outliers were omitted from this figure. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

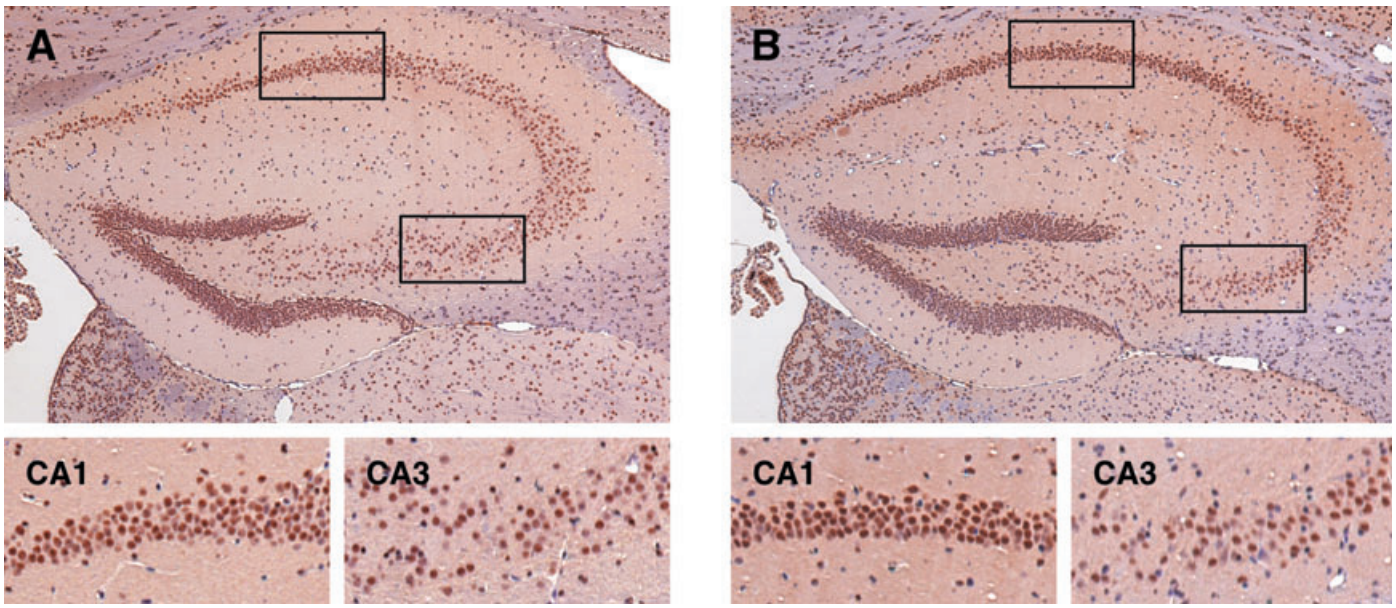

FIG. 3. Immunohistochemical assessment of glucocorticoid receptor expression in the murine hippocampus. (A) Hippocampal formation of a control mouse. A moderate-to-strong nuclear labelling of neurons is seen throughout all subfields of the hippocampus (top). The areas in boxes correspond to selected regions in subfields CA1 and CA3, and are shown at a higher magnification at the bottom. (B) Hippocampal formation of a scrapie-infected mouse. Note the almost identical staining pattern of neuronal nuclei in the whole hippocampal formation (top) as well as in the selected regions of CA1 and CA3 (bottom).

The main characteristics of chronic hyperactivity of the HPA axis have already been described independently and complementary for FFI and sheep scrapie (Portaluppi *et al*., 1994; Gayrard *et al*., 2000). To investigate if these mechanisms are also responsible for the increase in corticosterone metabolite levels in our murine scrapie model, other components of the HPA axis, particularly CRH and ACTH, will have to be analysed as soon as further noninvasive test systems are available. Concerning the influence of chronically elevated corticosteroid concentrations on GR expression in the hippocampus, our results are in agreement with those of other authors, who did not find relevant changes (Herman & Spencer, 1998; Herman *et al*., 1999), corroborating the hypothesis that (patho-) physiological elevations of glucocorticoids in chronic stress (even when hypercorticism is as severe as that seen in our scrapie model) might not be sufficient to downregulate GRs (Herman *et al*., 1999).

Apart from acute and chronic stress reactions, glucocorticoid secretion follows a circadian pattern of increase and decrease that is mainly regulated by two hypothalamic nuclei, the suprachiasmatic nucleus (acting as central circadian oscillator) and the paraventricular nucleus (Buijs & Kalsbeek, 2001). This diurnal variation is relatively independent of corticosteroid feedback inhibition in response to exogenous stimuli (Keller-Wood & Dallman, 1984). In our murine scrapie model, by investigation of circadian periodicity, we could discriminate two consecutive stages regarding the severity of changes. During the first phase, a circadian rhythm comparable to that in healthy animals dominated; however, despite this regular rhythm profile, a significant increase of faecal corticosterone metabolite excretion could already be detected at most time points in week −6, whereas the values of the 24-h pool samples were still normal at that time (Fig. 2 sampling week −6; Fig. 1). Thus, analysis of diurnal variation of hormone excretion offers a much more sensitive method of detecting early changes in the activity of the HPA axis. Towards the final disease phase, the circadian pattern was progressively disturbed, and, in the end, replaced by patterns that varied individually and were entirely irregular (Fig. 2 sampling week 0). One plausible explanation for these observations could be that during the first phase, the initial neurological symptoms caused by the prion disease (i.e. incipient disturbance of motor coordination) act as a stressor, which induces hyperactivity of the HPA axis. As this malfunction becomes chronic and deteriorates, it might lead to, and be, at the same time, perpetuated by further stress-related symptoms, in particular anxiety, restlessness, vertical leaping and, in advanced cases, a deregulated resting-activity cycle. The following complete loss of any regular circadian clock activity, might, eventually, be linked to the neurodegenerative process itself. Of note, the clinical picture elicited under such circumstances has to be distinguished from a Cushing-like syndrome, with a mere autonomous hypersecretion of corticosteroids resulting from tumours of the pituitary or adrenal gland. Accordingly, a Cushing-like phenotype has neither been observed in affected patients nor in our prion-infected animals (see Results).

A clinical relevance of an elevation of corticosteroids has already been postulated for other neurological disorders, such as Parkinson's and Alzheimer's disease in humans (Davis *et al*., 1986; Maeda *et al*., 1991; Hartmann *et al*., 1997; Peskind *et al*., 2001). In addition, a transgenic mouse model of Alzheimer's disease (Chishti *et al*., 2001) has recently been analysed by the same non-invasive technique as was used in the present study (Touma *et al*., 2004a). Female transgenic mice developed an approximately 1.8-fold rise in mean faecal corticosterone metabolite concentrations at the age of 120 days, shortly before the animals were killed (Touma *et al*., 2004a). In contrast, the scrapie inoculated female mice in our study showed significant elevation in 24-h pool samples over several weeks, culminating in a mean 4.3-fold increase. Taken together, abnormal activation of the HPA axis is not a specific feature of prion diseases, but rather represents a common secondary event in various neurodegenerative disorders, as has been described for other pathogenic mechanisms such as oxidative stress, complement activation, or activation of (pro-) apoptotic pathways (Giese *et al*., 1995; Guentchev *et al*., 2000; Kovacs *et al*., 2004). However, the great extent and the fast rate of increase of corticosteroid excretion observed in our study are striking, and have so far not been reported in any other human neurodegenerative disease or equivalent animal model. It appears

likely that this might influence the clinical picture, as it is well known that chronic hyperactivity of the HPA axis can have severe adverse effects on the central nervous system. In general, glucocorticoids regulate a wide variety of physiological functions by influencing the transcription of glucocorticoid-responsive genes. As a consequence, corticosteroid secretion is tightly regulated in mammals by negative-feedback loops. Chronic dysregulation of this control system with increased corticosterone secretion would have a profound impact on the transcriptional activity in several cell types, including neurons. In the hippocampus, sustained experimental elevation of corticosteroids caused disruption of synaptic plasticity, dendritic atrophy, shrinkage of nerve cell somata, nuclear pyknosis and, finally, overt nerve cell loss (Sapolsky *et al*., 1985, 1990; Sapolsky, 1999), all features that have been described in prion diseases (Belichenko *et al*., 2000; Brown *et al*., 2001; Jamieson *et al*., 2001). Recently, studies indicated participation of apoptotic pathways in neurons in response to increased corticosteroid concentrations (Almeida *et al*., 2000; Diem *et al*., 2003); of note, apoptosis was also identified as the primary cell death mechanism in prion diseases (for review see Unterberger *et al*., 2005). Furthermore, elevated corticosteroid concentrations increase the vulnerability of hippocampal neurons to oxidative stress (McIntosh *et al*., 1998; Reagan *et al*., 2000; Patel *et al*., 2002), the latter being known as an important event in prion diseases (Choi *et al*., 2000; Guentchev *et al*., 2000, 2002). Finally, it is important to consider that GRs are expressed widely throughout the brain across various species (Fuxe *et al*., 1985; Cintra *et al*., 1994; Morimoto *et al*., 1996; Sanchez *et al*., 2000). Therefore, increased corticosteroids could well act as a further cofactor in the neurodegenerative process, even in different types of prion diseases with their strain-specific neuropathological lesion profiles. One might imagine a scenario where elevation of corticosterone concentrations contributes to a structural and functional impairment of synapses and corticosteroid-sensitive neurons, eventually leading to nerve cell loss. This, in turn, could lead to a further deregulation of neuronal circuits and an increase in stress hormones, thereby constituting a fatal vicious circle that might participate in the rapid clinical decline of prion-affected individuals.

Supplementary material

The following supplementary material may be found on
http://www.blackwell-synergy.com

Fig. S1. Circadian pattern of corticosterone metabolite concentration of scrapie-infected and control mice before the onset of clinical symptoms.

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Abbreviations

ACTH, corticotropin; CRH, corticotropin-releasing hormone; EIA, enzyme immunoassay; FFI, fatal familial insomnia; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenocortical; MWU, Mann–Whitney *U*-test; PBS, phosphate-buffered saline; PrP^{C}, cellular isoform of the prion protein; PrP^{Sc}, disease associated isoform of the prion protein.

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