Corticoid Concentrations are Increased in the Plasma and Urine of Ewes with Naturally Occurring Scrapie

F. SCHELCHER, N. PICARD-HAGEN, V. LAROUTE, V. GAYRARD, M.A. POPOT, O. ANDREOLETTI AND P.L. TOUTAIN*


Abstract The 24-h pattern of corticoid plasma concentrations was determined in scrapie-affected ewes during the clinical phase of the disease. Twenty one ewes (8 healthy and 13 scrapie-affected ewes) were subjected to 24-h blood sampling periods. Urine samples were simultaneously obtained during the clinical stage of the disease and in healthy ewes. The scrapie diagnosis was performed by histopathology. Plasma and urinary corticoids were assayed using HPLC methods. Mean plasma and urinary levels of cortisol (cortisol, 20β-dihydrocortisol, cortisone) of scrapie-affected ewes were greater than those observed in healthy ewes. 20β-dihydrocortisol appeared to be the main cortisol metabolite in ewes. The intra-individual variations of 20β-dihydrocortisol plasma concentrations were lower than the corresponding plasma cortisol concentrations due to the dampening effect of the metabolic process on the short term variations of cortisol secretion. This dampening mechanism was amplified in urine, the urinary concentrations integrating cortisol production over the period preceding sampling. For these reasons, 20β-dihydrocortisol could present a potential interest for a non invasive diagnostic test of transmissible spongiform encephalopathies. The pathophysiological consequences of an excessive exposure to cortisol on development of the neurogenerative process are discussed.

Scrapie is an ovine sub-acute transmissible spongiform encephalopathy (TSE) caused by unconventional transmissible agents which include human Creutzfeldt-Jacob disease and Bovine Spongiform Encephalopathy. Currently, there is no preclinical diagnosis of TSE since infected animals lack immune responses. The definitive diagnosis is obtained by invasive methods including evidence of histopathological accumulation of the abnormal isofrom of PrP (PrPRes), identification of lesion markers or experimental demonstration of the transmissibility.

There is an urgent need to develop a specific, sensitive, practical and rapid diagnostic test for the detection of TSE. The exact nature of the etiologic agent remains unknown (10), and although the specific monoclonal antibody can discriminate the 2 isofroms of PrP (9), extensive basic research is still required to orient the development of a specific and sensitive diagnostic test directly related to the agent itself. In the absence of such tests, an indirect approach remains of value in early diagnosis.

Scrapie-induced brain damage could result in functional impairment as evidenced by electrophysiological measures in rodents (6,18) but this criterion is not practical for routine diagnosis. An alternative approach could consist of evaluating a blood and/or urinary marker of a dysfucntioning neuroendocrine system. Adrenal gland enlargement was found in ewes naturally affected with scrapie (3) and in experimentally infected hamsters (5). A disrupted pattern of cortisol secretion was observed in patients affected with the prion disease, fatal insomniania (16). This prompted us to investigate adrenal activity in scrapie-affected ewes.

Received 11/04/98.

* To whom all correspondence should be addressed

The purpose of this experiment was to examine the 24-h pattern of cortisol and its plasma metabolite concentrations and the urinary level of corticoids during the clinical phase of scrapie.

Materials and Methods

General. Two sets of experiments were performed. The first experiment was conducted with 11 ewes of Romanov breed: 5 healthy ewes and 6 ewes with naturally-contracted scrapie maintained under natural photoperiod. During the second experiment, 7 healthy ewes (5 of Romanov breed, 4 of which had been previously used, and 2 of Laacaune breed) and 7 ewes with naturally-contracted scrapie (3 of Romanov breed and 4 of Manchic breed) were kept in a light-sealed room under an artificial short photoperiod (9 h of light: 15 h of darkness). The ages of both healthy and diseased ewes ranged from one year and 6 months to 5 years. The diagnosis of scrapie, based on classical clinical signs, i.e., pruritus, behavioral changes, tremor and locomotor incoordination was established at least 10 days before the beginning of the experiments. The healthy ewes were included in the trial on the basis of an absence of clinical signs of scrapie. None of the classical clinical signs of scrapie has since been observed in these ewes during the 6 months following the sampling sessions. The ewes were individually tied in metabolism cages. They received daily rations of concentrate plus hay ad libitum and had free access to water.

Design. The objective of experiment I was to compare the temporal pattern of corticoid plasma concentrations in scrapie-affected ewes with that of healthy animals. A blood sampling session was performed after a 6-day period of acclimation to the experimental conditions. Peripheral blood samples were obtained from 11:00 the first day to 08:00 the next day, i.e. every 30 minutes or 3 hours. Experiment 2 aimed to examine the time course of corticoid levels during the clinical stages of scrapie.
Four 24-h blood sampling sessions separated by at least 10 days were carried out during about 2 months of the clinical phase of scrapie. The first session was performed after a 6-day period of acclimation to the experimental conditions. Peripheral blood samples were collected every 2-4 hours. The scrapie-affected ewes were sacrificed when they manifested the final clinical stages of scrapie, i.e., when the clinical signs had progressed to irreversible recumbency.

Blood sampling and assay. Blood samples were obtained from the left jugular vein with an indwelling catheter placed two hours before the onset of sampling sessions. Plasma was stored at -20°C until assayed. Plasma corticoid (cortisol, 20β-dihydrocortisol, cortisone) concentrations were determined using an a...
ewes varied from 3.5 to 27.5 ng/mL while those of healthy ewes varied from 2.5 to 3.5 ng/mL. Similarly, the overall mean plasma cortisone concentrations of scrapie-affected ewes ranged between 3.5 to 10.5 ng/mL, while those of healthy ewes ranged from 2.5 to 3.5 ng/mL.

![Graph showing cortisol concentrations in scrapie and healthy ewes](image)

**Fig. 2.** Overall mean plasma corticoid concentrations (±SD, ng/mL) observed during 24-h blood sampling periods carried out in scrapie-affected ewes during the clinical course of scrapie (dark area) and in healthy ewes (white area). The dashed line represents the limit of quantification of the assay, i.e. 2.5 ng/mL. Each bar indicates values obtained from one ewe.

**Corticoid urinary concentrations** The overall mean urinary cortisol concentrations of scrapie-affected ewes (80±59 ng/mL, 32-216 ng/mL) were significantly greater than those of healthy ewes (21±3 ng/mL, 20-29 ng/mL). As shown by the range of individual values, there were no overlapping cortisol urinary concentrations between healthy and scrapie-affected ewes. The mean urinary 20β-dihydrocortisone concentrations of scrapie-affected ewes (173±118 ng/mL, 34-430 ng/mL) were significantly greater than those of healthy ewes (29±16 ng/mL, 20-70 ng/mL). The mean urinary cortisone concentrations of scrapie-affected ewes (51±34 ng/mL, 20-122 ng/mL) were also significantly greater than those of healthy ewes (20±2 ng/mL, 20-25 ng/mL). Finally, the mean urinary 20β-dihydrocortisone concentrations of scrapie-affected ewes (61±36 ng/mL, 20-135 ng/mL) were significantly greater than those of healthy ewes (22±4 ng/mL, 20-32 ng/mL). For cortisol metabolites, the range of individual values was increased but there was some overlapping between urinary concentrations from healthy and scrapie-affected ewes. There was no effect of sampling period on urinary corticoid concentrations.

**Histology.** The diagnosis of scrapie was confirmed by the identification of perikaryonic and/or neuropil vacuolisation in at least three grey matter nuclei. Adrenal gland examination showed evidence of hypertrophy of the cells of the zona fasciculata of the adrenal cortex.

**Discussion**

The main result of the present study is that plasma and urinary corticoid levels were significantly increased in scrapie-affected ewes, suggesting that this prion disease displays a syndrome of hypercortisolism. The increase in plasma and urinary corticoid concentrations was associated with hypertrophy of the adrenal cortex in scrapie-affected animals.

In our experiment, most of the plasma sample levels of cortisol in healthy ewes were within the range of physiological values (7). A nycthemeral rhythm of cortisol secretion was not evidenced in either healthy or scrapie-affected ewes. In scrapie-affected ewes, cortisol plasma levels were significantly increased and reached values greater than those normally attained at the peak of the 24-h plasma profile reported in some studies (13), in response to a stimulus like daily feeding (17) or under certain physiological conditions such as pregnancy (4). Hypercortisolism could be a specific endocrine feature of the prion disease as no sign of stress was observed in the scrapie-affected ewes and most of the samples were obtained while clinical signs of the disease were still subtle. In contrast, it was demonstrated that the chronic pain associated with lameness resulted in a decrease in plasma cortisol concentrations instead of inducing an increase (11). In the context of the research for a precocious marker of scrapie, it would be of great interest to date the occurrence of the disrupted adrenal function during the preclinical phase of the naturally occurring disease in ewe.

Such a syndrome of hypercortisolism of scrapie-affected ewes could be relevant for the further development of a diagnostic test of the prion disease. However, cortisol plasma concentrations are subject to fluctuations of endogenous origin (pulsatility or possible diurnal rhythm) or exogenous (e.g. in response to a stimulus such as the acute stress of the sampling procedure). This means that the peak cortisol plasma levels of healthy ewes can be higher than the trough levels of scrapie-affected ones, thereby impeding the potential use of cortisol as a diagnostic scrapie marker. This drawback does not exist for cortisol metabolites because the slow process of metabolism acts as a damping and delaying mechanism resulting in less temporal variations of individual plasma cortisol metabolite levels. A dampening mechanism also exists for urine since urinary cortisol levels reflect hormone production over the period between the previous spontaneous urine emission and the moment of urine sampling. For these reasons, urine cortisol or the metabolites of cortisol (plasma, urine) are potential biological markers of altered adrenal...
function and 20β-dihydrocortisol, which is apparently the main cortisol metabolite, is probably the best analyte to discriminate between healthy and diseased ewes.

Stimulation of the adrenocortical function in scrapie-affected ewes is consistent with the abnormal enlargement of the adrenal glands already observed in ewes naturally affected with scrapie (3) and in experimentally infected mice (8) and hamsters (5,21). From a mechanistic point of view, the stimulation of adrenal activity in scrapie-affected ewes could result from specific alterations of the neuronal systems involved in control of the hypothalamic-pituitary-adrenal axis or from autonomous hyperactivity of the adrenal gland of infected animals. The former hypothesis is the more probable since a functional hypertrophy of the suprachiasmatic-infundibular neurosecretory subsystem was evidenced in ewes naturally affected with scrapie (15). More recently, an increase in the number of CRF neurons was observed in the hypothalamus of scrapie-affected hamsters (20).

Whatever the origin of adrenal hyperactivity, attention should be paid to the pathophysiological meaning and consequences of chronic exposure to high levels of corticoid. Indeed, glucocorticoids were shown to have protective effects by slowing the rate of Alzheimer’s disease progression since the activation of specific inflammatory mechanisms contributes to neurodegeneration (1). Recent studies suggest that a glial production of cytokine induced by PrPsc deposition may contribute to the development of pathological lesions in a murine scrapie model (19). An inflammatory nature of the brain lesions could explain the reduced susceptibility to scrapie induced by steroid administration to experimentally infected mice (14). On the other hand, a long term sustained secretion of glucocorticoids could exacerbate the neurodegenerative process characterizing the prior diseases. Indeed, excessive exposure to glucocorticoids has adverse effects on the rodent brain regions possessing high concentrations of glucocorticoid receptors (12).

In conclusion, we have demonstrated that plasma and urinary concentrations of corticoids were much higher in scrapie-affected ewes than in healthy ewes. Our results suggest that 20β-dihydrocortisol merits special attention as a potential biological marker of scrapie disease since the plasma 20β-dihydrocortisol levels appeared relatively stable throughout the 24 hours.

References
12. McIntosh LJ, Sapolisky RM 1996 Glucocorticoids may enhance oxygen radical-mediated neurotoxicity. Neurotoxicology 17:873-882