

# Plasma profiles of adrenocorticotrophic hormone, cortisol, $\alpha$ -melanocyte-stimulating hormone, and growth hormone in dogs with pituitary-dependent hyperadrenocorticism before and after hypophysectomy

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## Abstract

The 6-h plasma profiles of adrenocorticotrophic hormone (ACTH), cortisol,  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH), and GH were studied in 17 dogs with pituitary-dependent hyperadrenocorticism (PDH) before and after hypophysectomy. The aim of the study was to investigate the relation between the hormone profile characteristics and recurrence of PDH after surgery.

The hormones were secreted in a pulsatile fashion. The basal plasma cortisol concentration and area under the curve (AUC) for cortisol were significantly higher in the PDH cases than in eight controls. The characteristics of the plasma profiles of ACTH and  $\alpha$ -MSH were not significantly different between the PDH cases and the controls. In the PDH cases, less GH was secreted in pulses than in the controls, but the difference was not significant. The basal plasma cortisol concentration, the AUC for ACTH and cortisol, and the pulse frequency of ACTH and cortisol decreased significantly

after hypophysectomy for the group of PDH cases. The basal plasma concentrations of ACTH and  $\alpha$ -MSH, the AUC for  $\alpha$ -MSH, and the characteristics of the plasma GH profiles of the PDH cases remained unchanged after hypophysectomy. No pulses of  $\alpha$ -MSH were observed after hypophysectomy. The co-occurrence between the ACTH and cortisol pulses decreased significantly with hypophysectomy. The post-operative pulse frequency of ACTH was the only characteristic with predictive value for the recurrence of PDH after hypophysectomy.

The results of this study demonstrate that ACTH, cortisol,  $\alpha$ -MSH, and GH are secreted in a pulsatile fashion in dogs with PDH. Hypophysectomy effectively reduces the secretion of ACTH and cortisol. The presence of ACTH pulses after hypophysectomy is a risk factor for the recurrence of hyperadrenocorticism.

*Journal of Endocrinology* (2006) **190**, 601–609

## Introduction

Pituitary-dependent hyperadrenocorticism (PDH) is a common spontaneous endocrine disorder in dogs, which shows many similarities with Cushing's disease in humans (Kempainen & Peterson 1994). As in humans with Cushing's disease (Lamberts 2002, Reimondo *et al.* 2003), PDH in dogs is characterized by adrenocorticotrophic hormone (ACTH)-induced hypercortisolism and reduced sensitivity to glucocorticoid feedback inhibition (Rijnberk *et al.* 1988a, Bosje *et al.* 2002), in most cases with preserved ability to respond to corticotrophin-releasing hormone (CRH) stimulation (Meij *et al.* 1997b, Reimondo *et al.* 2003). The excessive pituitary secretion of ACTH originates from a corticotrophic adenoma in the pars distalis or the pars intermedia of the adenohypophysis. PDH caused by an adenoma in pars intermedia is often characterized by highly elevated plasma concentrations of  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH) and strong

resistance to dexamethasone suppression (Peterson *et al.* 1986, Rijnberk *et al.* 1988b, Bosje *et al.* 2002). In Cushing's disease, there are not only alterations in ACTH secretions but also in the secretion of other pituitary hormones (van den Berg *et al.* 1997, Roelfsema *et al.* 1998, Veldman *et al.* 2000). In dogs with PDH, these changes may include basal levels, response to stimulation (Meij *et al.* 1997b), as well as the pulsatile release pattern that is characteristic of pituitary hormone secretion (Kempainen & Sartin 1984, Brabant *et al.* 1992, Kooistra *et al.* 1997a, 1999, Kooistra & Okkens 2001, Corrada *et al.* 2003, Lee *et al.* 2003). For example, plasma growth hormone (GH) concentration and its responsiveness to stimulation with GH-releasing hormone (GHRH) have been reported to be decreased in dogs with PDH, most likely due to glucocorticoid-induced alterations in the function of pituitary somatotrophic cells and changes in suprapituitary regulation (Meij *et al.* 1997b). In addition, canine PDH is associated with less GH secreted in pulses than in controls (Lee *et al.* 2003).

Pituitary surgery is the treatment of choice in humans with Cushing's disease (Rees *et al.* 2002). Preferably, an adeno-mectomy is performed, but 15–26% of the cases undergo a total hypophysectomy (Barker *et al.* 2003). Transsphenoidal hypophysectomy is an effective treatment in dogs with PDH (Meij *et al.* 1998). In both species, recurrence of the disease is a serious problem. In a study on 150 dogs with PDH, the fraction that relapsed within 2 years after hypophysectomy was 25% (Hanson *et al.* 2005). In humans, the recurrence rate is 5–30% after pituitary surgery for corticotropic adenomas (Rees *et al.* 2002, Pereira *et al.* 2003).

After transsphenoidal hypophysectomy in healthy dogs, residual pituitary cells have been observed in the pituitary fossa (Niebauer *et al.* 1990, Meij *et al.* 1997d, Axlund *et al.* 2005). Such cells are devoid of the direct influence of the hypothalamus due to section of the pituitary stalk. Upon administration of secretagogues, the residual corticotropic cells appeared functional. They secreted ACTH after stimulation with CRH (Meij *et al.* 1997a). Stimulation with other secretagogues, e.g. thyrotropin-releasing hormone (TRH), GHRH, and gonadotropin-releasing hormone (GnRH), evoked no adeno-hypophyseal hormone secretion (Meij *et al.* 1997d). Eight weeks after surgery, the CRH stimulation test was not able to identify the dogs that would later relapse (Meij *et al.* 1997a). The pulsatile secretion pattern of remnant pituitary cells has not yet been studied. It was hypothesized that the secretion pattern would differ between remnant adenomatous and normal corticotropic cells and that the profile characteristics after hypophysectomy would hold predictive value for recurrence of hyperadrenocorticism.

Here, we report on the 6-h plasma profiles of ACTH, cortisol,  $\alpha$ -MSH, and GH in dogs with PDH before and after transsphenoidal hypophysectomy. The aim of the study was to investigate the relation between the hormone profile characteristics and recurrence of PDH after surgery.

## Materials and Methods

### *Animals, diagnosis, and treatment of PDH*

Seventeen dogs of different breeds with PDH were included in the study. The group comprised two Miniature Poodles, one Dachshund, one Basset Fauve de Bretagne, one Cairn Terrier, one English Cocker Spaniel, one Pitt Bullterrier, one Shetland Sheepdog, one Soft Coated Wheaten Terrier, one Standard Poodle, one Welsh Corgi and six cross-bred dogs. There were 6 females (2 spayed) and 11 males (2 castrated) with a median age of 8 years (range 5–12 years), and a median body weight of 15 kg (range 7–27 kg).

The diagnosis of hyperadrenocorticism was based upon averaged urinary corticoid-to-creatinine ratios (UCCR) in two consecutive morning urine samples. In all animals, the UCCRs (median,  $44 \times 10^{-6}$ ; range,  $23$ – $321 \times 10^{-6}$ ) exceeded the ratios found in 87 healthy companion dogs (range  $0.3$ – $8.3 \times 10^{-6}$ ; van Vonderen *et al.* 1997). After

collection of the second urine sample, three oral doses of  $0.1$  mg dexamethasone per kilogram body weight were administered at intervals of 8 h. In 13 cases, the UCCR in the third sample was less than 50% of the mean of the first two samples and PDH was diagnosed (Galac *et al.* 1997). In four cases, with less than 50% suppression of the third UCCR, pituitary dependency was secured by measurements of plasma ACTH concentrations and further supported by visualization of the adrenals by ultrasonography and by pituitary imaging with computed tomography (CT; Rijnberk *et al.* 1987, Bosje *et al.* 2002, van der Vlugt-Meijer *et al.* 2002, 2003).

Transsphenoidal hypophysectomy was performed according to a microsurgical technique described previously (Meij *et al.* 1997c). Postoperative care and hormone supplementation were according to previously published protocols (Meij *et al.* 1997c, 1998, Hanson *et al.* 2005). Briefly, hydrocortisone and desmopressin were directly administered after the removal of the pituitary gland. When the dogs had resumed drinking and eating, oral substitution therapy was started with cortisone acetate and thyroxine. The dose of cortisone acetate was gradually lowered over a period of 4 weeks to a physiological dose. Desmopressin was administered for 2 weeks routinely and continued if polyuria due to central diabetes insipidus persisted.

Re-examination after 8 weeks included physical examination, routine blood chemistry, measurements of basal plasma thyroxine concentration at 10–12 h after L-thyroxine medication, and basal UCCR in duplicate at 24 h after cortisone medication (median  $1.7 \times 10^{-6}$ ; range  $0.3$ – $5.2 \times 10^{-6}$ ). UCCRs were measured again half a year after surgery and thereafter once a year. In case of suspicion of recurrence, UCCRs were determined earlier. Urine samples were mailed to our laboratory and follow-up reports were obtained from the above-mentioned routine follow-up examinations in the hospital and during telephone conversations with the owner and/or the referring veterinarian.

There was one case of early postoperative mortality (within 4 weeks) due to kidney failure. In all 16 remaining dogs, there was remission: UCCR  $< 10 \times 10^{-6}$  and resolution of clinical signs of hyperadrenocorticism. Recurrence was defined as UCCR  $\geq 10 \times 10^{-6}$  and/or return of signs and symptoms of hyperadrenocorticism after initial complete remission. This occurred in 9 out of the 16 cases after median 652 days (range 201–1679 days).

### *Control dogs for pulsatile secretion*

The plasma profiles of cortisol, ACTH, and  $\alpha$ -MSH of the PDH cases were compared with those obtained in eight healthy beagle dogs (four intact females and four intact males, with body weight ranging from 12 to 25 kg, and age ranging from 2 to 5 years; Kooistra *et al.* 1997a). The plasma GH profiles of the PDH cases were compared with those of plasma obtained from six healthy female beagle dogs (with body weight ranging from 12 to 27 kg, and age ranging from 7 to 9 years; Lee *et al.* 2003).

### Sample collection for pulsatile plasma profile

In the PDH dogs, the 6-h plasma hormone profiles were determined 2–34 days (median, 8 days) before surgery and 50–133 days (median, 71 days) after hypophysectomy. Preoperative 6-h plasma profiles of ACTH and cortisol were available from 17 cases,  $\alpha$ -MSH from 14 cases, and GH from 14 cases. After hypophysectomy, 6-h plasma profiles of ACTH and cortisol became available from 14 cases,  $\alpha$ -MSH from 13 cases, and GH from 11 cases.

Food, but not water, was withheld from the animals 12 h prior to blood sampling. Blood samples (4 ml) were collected from the jugular vein by an experienced technician at 10-min intervals between 0800 and 1400 h. Blood was collected in pre-chilled EDTA-coated tubes on ice and centrifuged at 4 °C (2000 g, 10 min). Plasma was stored at –20 °C until assayed.

This study on the pulsatile secretion was approved by the Ethical Committee of Utrecht University and for the PDH dogs informed consent was obtained from the owners.

### Hormone determinations

Plasma ACTH concentration was measured using a commercially available two-site immunoradiometric assay (IRMA; Nichols Institute, Wijen, The Netherlands). The antiserum was highly specific for ACTH (1–39). A polyclonal antibody was bound specifically to the C-terminal region of ACTH. The radioiodinated monoclonal antibody was bound only to the N-terminal region of ACTH. The intra-assay coefficient of variation (CV) was 3.2%, the inter-assay CV was 7.8%, and the sensitivity was 0.22 pmol/l. There was no cross-reaction between the antiserum and  $\alpha$ -MSH or ACTH precursors (Raff & Findling 1989, Bosje *et al.* 2002).

Plasma cortisol concentration was measured with a solid phase <sup>125</sup>I RIA; Coat-A-Count Cortisol, Diagnostic Products Corporation, Los Angeles, CA, USA. The antiserum was highly specific for cortisol, with very low cross-reactivity to other compounds that were present in patient samples. Proteins, bilirubin, lipemia, and hemolysis had no significant effect on the assay. The intra-assay CV was 4.5–6.3%, the inter-assay CV, 4%, and the sensitivity, 5.5 nmol/l.

The urinary corticoid concentration was measured with RIA as described previously (Rijnberk *et al.* 1988a). The intra-assay CV was 6%, the inter-assay CV, 8%, and the sensitivity, 1 nmol/l. The urinary corticoid concentration was related to the urinary creatinine concentration (Jaffé kinetic method, initial rate reaction) and the UCCR calculated (Stolp *et al.* 1983, Rijnberk *et al.* 1988a).

Plasma concentration of  $\alpha$ -MSH was measured with RIA without extraction as described previously (Rijnberk *et al.* 1988b). The intra-assay CV was 10%, the inter-assay CV, 23%, and the sensitivity, 3 pmol/l. The antiserum had less than 0.1% cross-reactivity with ACTH (1–39) and 4% cross-reactivity with ACTH (1–24).

Plasma GH concentration was measured by a homologous RIA as described previously (Eigenmann & Eigenmann 1981). The intra-assay CV was 3.8%, the inter-assay CV, 7.2%, and the sensitivity, 0.3 µg/l. The degree of cross-reaction of canine prolactin was 2%.

### Statistical analysis

The 6-h plasma hormone profiles were analyzed using the Pulsar program developed by Merriam & Wachter (1982). The program identifies secretory peaks by height and duration from a smoothed baseline, using the assay S.D. as a scale factor. The cut-off parameters G1–G5 of the Pulsar program were set at 3.98, 2.4, 1.68, 1.24, and 0.93 times the assay S.D. as criteria for accepting peaks 1, 2, 3, 4, and 5 points wide. The smoothing time, a window used to calculate a running mean value, was set at 5 h. The weight assigned to peaks was 0.05. The A, B, and C values of the Pulsar program used to calculate the variance of the assay was set at A=0, B=7.1, and C=32 for ACTH; A=0, B=4.8, and C=114 for cortisol; A=0, B=–2.44, and C=451 for  $\alpha$ -MSH; and A=0, B=7.2, and C=5 for GH. The values extracted from the Pulsar analyses included the overall mean of the smoothed baseline, area under the curve (AUC) above the zero level, and the number of significant pulses per 6 h.

The differences in variables between control dogs and dogs with PDH were assessed by the non-parametric Mann–Whitney test (with Bonferroni correction). The differences in variables before and after surgery were assessed by Wilcoxon–signed ranks test for related samples (with Bonferroni correction). A  $P < 0.05$  was considered significant. Fisher's exact test was used to analyze the co-occurrence of significant ACTH, cortisol, and  $\alpha$ -MSH pulses.

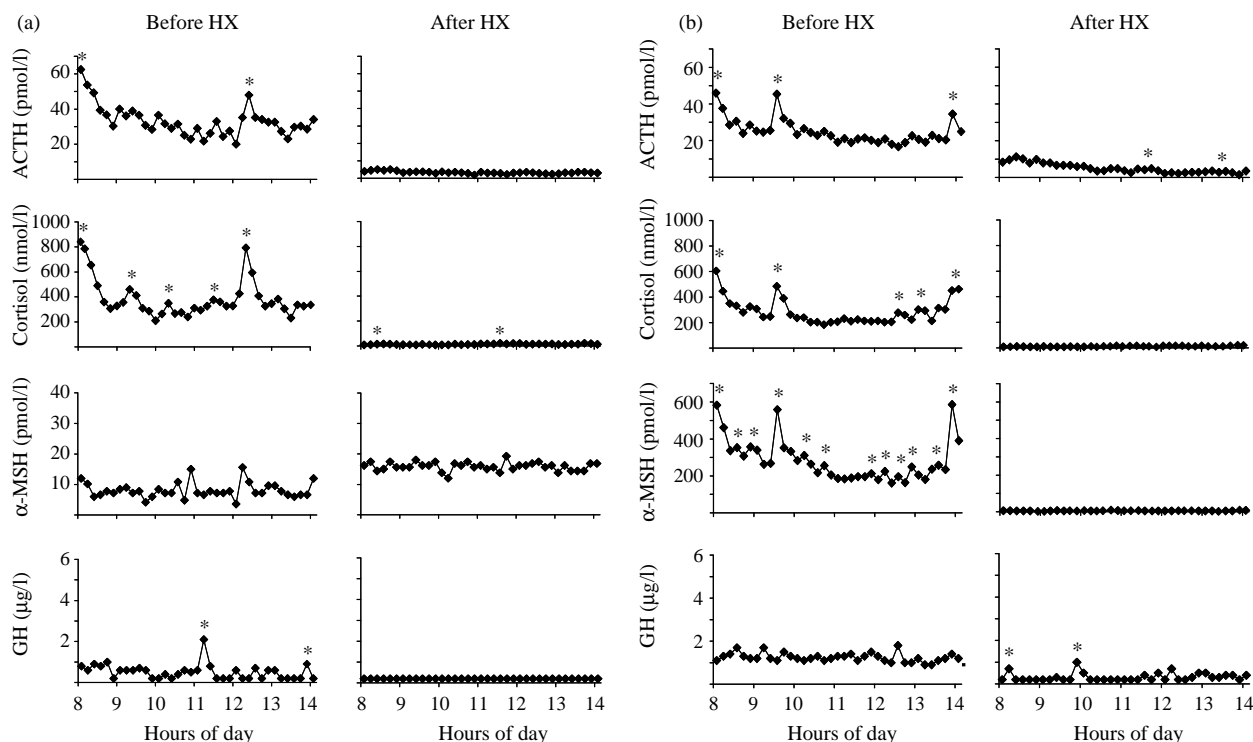
Univariate Cox proportional hazard fit analyses were performed using Newton–Raphson algorithm for the disease-free period on the characteristics of the plasma hormone profiles (mean of the smoothed baseline, AUC, pulse frequency) for ACTH, cortisol,  $\alpha$ -MSH, and GH before and after hypophysectomy.

## Results

All four hormones were secreted in a pulsatile fashion in control dogs as well as in dogs with PDH (Fig. 1a and b). Representative graphs of the plasma profiles for the controls have been published previously (Kooistra *et al.* 1997a, Lee *et al.* 2003).

### ACTH and cortisol

In the dogs with PDH, the basal plasma cortisol concentration ( $P < 0.005$ ) and the AUC for cortisol ( $P < 0.001$ ) were significantly higher than those in the controls. There was no significant difference in the cortisol pulse frequency. After hypophysectomy, the basal plasma



**Figure 1** The plasma profiles of ACTH, cortisol,  $\alpha$ -MSH, and GH before and after hypophysectomy (HX) in (a) a representative dog with pituitary-dependent hyperadrenocorticism (PDH), and (b) a dog with PDH with markedly elevated plasma  $\alpha$ -MSH concentrations before hypophysectomy (note the difference in scale of the y-axis 1b). Blood samples were collected at 10-min intervals for 6 h. \*Peaks identified by the Pulsar programme.

cortisol concentration ( $P < 0.005$ ), the AUC for cortisol ( $P < 0.005$ ), and the cortisol pulse frequency ( $P < 0.005$ ) decreased significantly (Fig. 2). The basal plasma ACTH concentration, the AUC for ACTH, and the ACTH pulse frequency in the PDH dogs were not significantly different from those in the controls. Significant ACTH pulses were identified by the Pulsar program in 14 out of the 17 dogs with PDH. After surgery, the AUC for ACTH ( $P < 0.05$ ) and the ACTH pulse frequency ( $P < 0.05$ ) decreased significantly, whereas the basal plasma ACTH concentration tended to decrease ( $P = 0.052$ ) (Fig. 2). Significant ACTH pulses were identified in 10 out of the 14 cases after hypophysectomy; 8 of these 10 dogs had a recurrence later. Significant ACTH pulses were absent in 4 out of the 14 cases and 3 of these 4 dogs remained in remission.

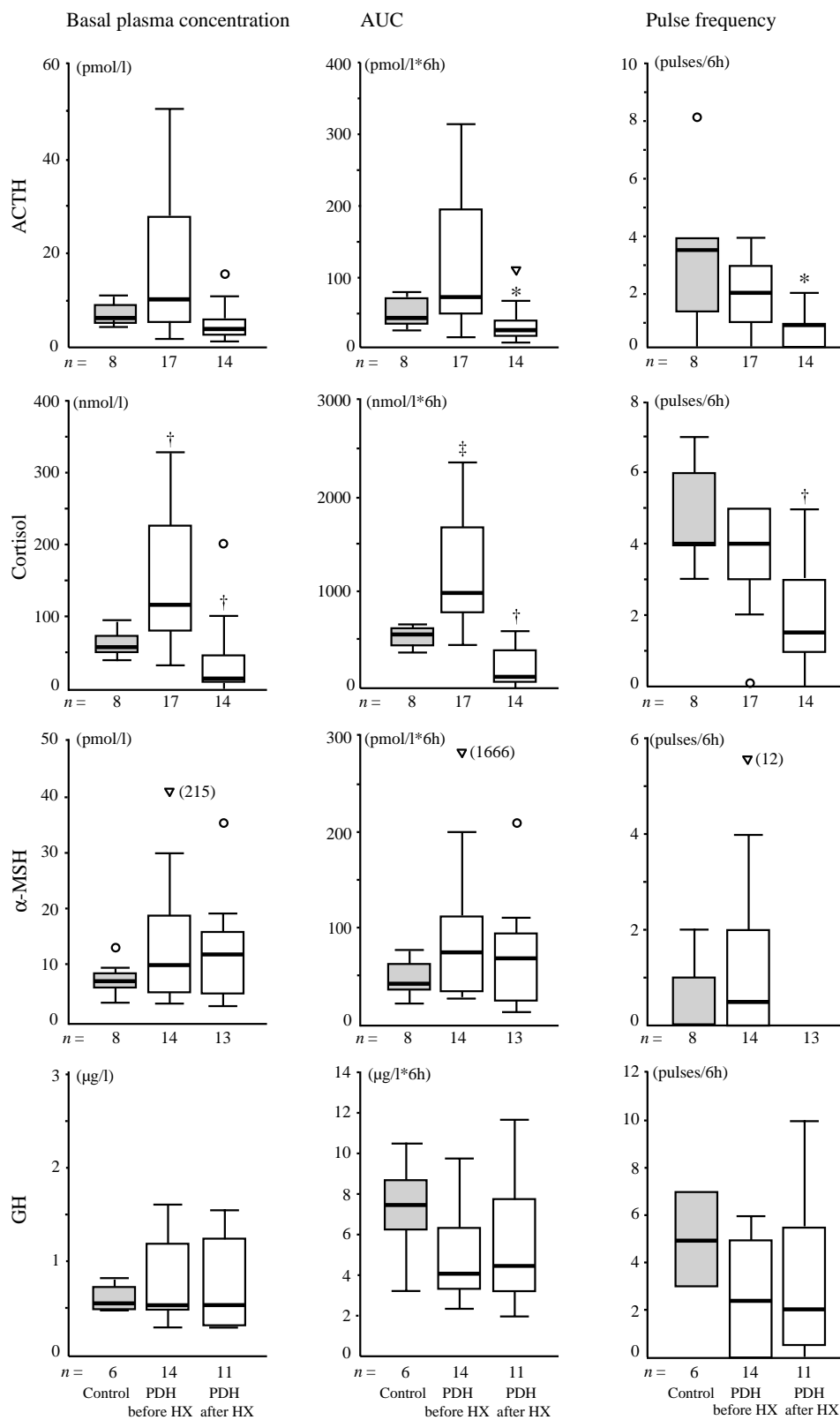
In the PDH dogs and in the controls, the majority of ACTH pulses coincided with cortisol pulses. In the controls, 23 out of 26 significant ACTH pulses identified by the Pulsar

program coincided with 21 out of 38 significant cortisol pulses. In the PDH cases, 32 out of 36 significant ACTH pulses coincided with 32 out of 65 significant cortisol pulses. The difference between co-occurrence of ACTH and cortisol pulses in control and PDH cases was not significant. After surgery, 5 out of 12 significant ACTH pulses coincided with 5 out of 26 significant cortisol pulses. The difference between co-occurrence of ACTH and cortisol pulses before and after surgery was significant ( $P < 0.01$ ).

#### $\alpha$ -MSH

The basal plasma  $\alpha$ -MSH concentration, the AUC for  $\alpha$ -MSH, and the  $\alpha$ -MSH pulse frequency in the PDH cases were similar to those in the controls. Significant  $\alpha$ -MSH pulses were identified in 7 out of the 14 PDH cases. After hypophysectomy, no significant  $\alpha$ -MSH pulses were detected in any of the dogs. In one dog with dexamethasone-resistant

**Figure 2** Box-plot graphs (median, interquartile and total range) for the basal plasma hormone concentration, the area under the curve (AUC), and the pulse frequency of ACTH, cortisol,  $\alpha$ -MSH and GH in control dogs and in dogs with pituitary-dependent hyperadrenocorticism (PDH) before and 2–4 months after hypophysectomy (HX). Blood samples were collected at 10-min intervals for 6 h. Outliers are indicated with circles; extreme values with triangles. For values that are off scale the actual value is given within brackets. Significant differences between groups are indicated with asterisks above the boxplots. Asterisks above the boxes for PDH dogs before HX indicate differences compared with the control dogs and asterisks above the boxes for PDH dogs after HX indicate differences compared with PDH dogs before HX; \*  $P < 0.05$ ; †  $P < 0.01$ ; ‡  $P < 0.001$ .



PDH, a very high basal plasma  $\alpha$ -MSH concentration (215 pmol/l; reference range  $\leq 36$  pmol/l (Kooistra *et al.* 1997b, Bosje *et al.* 2002)) was found and 12 significant  $\alpha$ -MSH pulses identified (Fig. 1b). In this case, the postoperative basal  $\alpha$ -MSH concentration was 4.5 pmol/l (Fig. 1b).

In the PDH dogs and in the controls  $\alpha$ -MSH pulses frequently coincided with ACTH pulses. In the controls, 3 out of 4 significant  $\alpha$ -MSH pulses coincided with significant ACTH pulses, whereas in the PDH cases, 9 out of 25 significant  $\alpha$ -MSH pulses coincided with significant ACTH pulses. In the dog with 12 significant  $\alpha$ -MSH pulses, only 3 (high)  $\alpha$ -MSH pulses coincided with a significant ACTH pulse (Fig. 1b). In some cases, an  $\alpha$ -MSH pulse preceded an ACTH pulse.

## GH

The basal plasma GH concentration, the AUC for GH, and the GH pulse frequency were not significantly different between controls and PDH dogs, and also not between the PDH cases before and after hypophysectomy. Significant GH pulses were detected in 11 out of 15 dogs with PDH. After hypophysectomy, significant GH pulses were detected in 8 out of the 11 PDH cases.

## Disease-free period and identification of risk parameters

The median disease-free period was 880 days (95% CI, 732–1028 days). The 1-year disease-free fraction was 87% (95% CI, 56–96%) and the 2-year disease-free fraction, 65% (95% CI, 35–84%). In 9 out of the 16 cases, hyperadrenocorticism recurred after 1.5–5.5 years. In the univariate Cox proportional hazard analysis, there was no association between preoperative hormone values and recurrence of hyperadrenocorticism. Of the values after hypophysectomy, the ACTH pulse frequency was associated with a significant ( $P < 0.05$ ) higher risk for recurrence of hyperadrenocorticism (hazard ratio 5.357; 95% CI, 1.003–28.611). A higher AUC for GH after surgery tended to be associated with a lower risk of recurrence ( $P = 0.076$ ; hazard ratio 0.768, 95% CI, 0.553–1.066).

## Discussion

The results of this study demonstrate that the pulsatile nature of the pituitary hormone release is maintained in pituitary-dependent hyperadrenocorticism. Significant pulses of ACTH (and consequently cortisol),  $\alpha$ -MSH, and GH were identified in the plasma profiles of dogs with PDH. Interestingly, significant pulses were also observed in some of the cases in remission after hypophysectomy. The presence of significant ACTH pulses after surgery was identified as a risk factor for recurrence of hyperadrenocorticism.

Before surgery, the pulse frequencies of ACTH and cortisol in the PDH cases were not different from those in the

controls, which is in agreement with previous reports on dogs (Orth *et al.* 1988) and on humans with Cushing's disease (van den Berg *et al.* 1995). In this study, the AUC for ACTH and ACTH pulse frequency decreased after hypophysectomy. In addition, the basal plasma cortisol concentration, the AUC for cortisol, and the cortisol pulse frequency decreased significantly and there was less co-occurrence between ACTH and cortisol pulses after surgery. In accordance with the relatively low values of the plasma profile characteristics for ACTH and cortisol after hypophysectomy, remission was achieved in all 16 dogs that survived the early postoperative period.

In this study, complete hypophysectomy with elimination of all ACTH-producing cells was the surgical goal. Hypophysectomy is consistent with a substantial reduction in the number of corticotrophic cells, which leads to remission of hyperadrenocorticism. Nevertheless, there was residual ACTH secretion after hypophysectomy. Corticotrophic cells are fairly resistant to elimination and complete removal of the pituitary gland is difficult to achieve (Ganong & Hume 1956). Also, in what macroscopically appears to be an empty pituitary fossa, small remnant microscopic islets of pituitary cells capable of hormone production and response to CRH stimulation test have been found after hypophysectomy in both controls and PDH dogs (Niebauer *et al.* 1990, Meij *et al.* 1997a, 1997d, Axlund *et al.* 2005). The most likely origin of these cells are remnants from an incompletely removed pars distalis hypophysis (which easily falls apart upon manipulation) or, in the PDH cases, remnant adenomatous corticotrophic cells. Less likely, the remnant cells may also originate from differentiated pituitary stem cells or from accessory pituitary tissue that, in the dog, is sporadically found in the dura mater lateral to the pituitary (von Nickel *et al.* 1992).

In previous reports, no remnant pituitary cells were identified on the ventral hypothalamic diencephalons in hypophysectomized experimental animals (Meij *et al.* 1997a, 1997d, Axlund *et al.* 2005). Therefore, it can be assumed that the pituitary-hypothalamic portal system is completely impeded and that the residual hormone secretion activity measured in this study originates from the microscopic islets in the pituitary fossa. Interestingly, the residual ACTH secretion was pulsatile in character. It is possible that this could be due to the influence of CRH, to which the corticotrophic cells in the pituitary residual islets are able to respond (Meij *et al.* 1997a, 1997d), reaching the pituitary cells on an alternative vascular route or through the cerebrospinal fluid from the opened third ventricle after hypophysectomy. The residual pulsatile ACTH secretion after hypophysectomy may also be explained by intrinsic ACTH pulsatility (Gambacciani *et al.* 1987), by release of vasopressin from the hypothalamus or by CRH-independent secretory capacity of corticotrophic cells (Fukuda *et al.* 2004).

In agreement with earlier observations (Orth *et al.* 1988, Kooistra *et al.* 1997a),  $\alpha$ -MSH was secreted in a pulsatile fashion in both the controls and the PDH dogs. Under basal conditions, the pars intermedia of the canine



adenohypophysis is under strong and almost permanent dopaminergic inhibition (Peterson *et al.* 1986, Kemppainen & Sartin 1988, Orth *et al.* 1988, Meij *et al.* 1997d). The significant  $\alpha$ -MSH pulses are probably the result of a temporary decrease in the tonic hypothalamic dopaminergic inhibitory control. The administration of a dopamine antagonist such as haloperidol results in a significant release of  $\alpha$ -MSH (Orth *et al.* 1988, Kooistra *et al.* 1997a).

The  $\alpha$ -MSH pulsatility was similar in the PDH dogs and the controls. After hypophysectomy, there were no  $\alpha$ -MSH pulses. This is compatible with complete removal of the neurointermediate lobe. In a previous study on pituitary function after hypophysectomy in experimental animals, administration of the dopamine-antagonist haloperidol caused no elevation of the basal plasma  $\alpha$ -MSH concentration, whereas before surgery there was a 30-fold increase (Meij *et al.* 1997d).

Several  $\alpha$ -MSH pulses co-occurred with ACTH pulses that is explained by co-release of ACTH and  $\alpha$ -MSH from the pars intermedia (Kooistra *et al.* 1997a) by the same secretagog or stimulatory event. Previous studies demonstrated that the dopamine-antagonist haloperidol increases the plasma concentrations of both  $\alpha$ -MSH and ACTH (Kemppainen & Sartin 1988, Kooistra *et al.* 1997a). The haloperidol-stimulated secretion of  $\alpha$ -MSH reached its maximum value within 10 min, whereas the haloperidol-stimulated secretion of ACTH reached its maximum value after 60 min. Therefore, an  $\alpha$ -MSH pulse preceded an ACTH pulse in some cases of the present study.

Dogs with PDH and humans with Cushing's disease secrete less GH in pulses than healthy individuals (Veldman *et al.* 2000, Lee *et al.* 2003). The same was observed in the present study, although no statistical significance was reached. Sustained exposure to supraphysiological amounts of glucocorticoids inhibits pulsatile GH secretion and blunts the GH response to stimuli mainly by altering the hypothalamic somatostatin tone (Muller *et al.* 1999).

In the group of PDH dogs, hypophysectomy caused no further significant decrease in the already low plasma GH concentrations. However, in some individual cases, the decline of the basal GH values was distinct. These findings are in agreement with a previous study in which hypophysectomy led to a significant decrease in the plasma GH concentrations (Meij *et al.* 1997a). Plasma GH concentrations unaffected by hypophysectomy are most likely the result of secretion by residual somatotrophic cells in the pituitary fossa (Hassan & Merkel 1994, Meij *et al.* 1997a, 1997d). Another explanation is the extra-pituitary GH production that occurs in many tissues under both normal and pathologic conditions (Mol *et al.* 1995, van Garderen *et al.* 1997, Lantinga van Leeuwen *et al.* 2000, Harvey *et al.* 2001, 2004, Robben *et al.* 2002, Petterino *et al.* 2004). This extra-pituitary GH is mainly thought to have local autocrine and paracrine effects, but may also reach the systemic circulation. For example, progesterone-induced GH release from the mammary gland during the luteal phase may increase the plasma GH

concentration in bitches (Kooistra & Okkens 2002). However, hypophysectomy removes the FSH- and LH-secreting gonadotropic cells (resulting in permanent anoestrus and a very low plasma progesterone concentration), and therefore, it is unlikely that circulating GH after hypophysectomy is of mammary origin.

There was no association between preoperative characteristics of the plasma hormone profiles and recurrence of hyperadrenocorticism, which is in agreement with the results of a study after transsphenoidal hypophysectomy in humans with Cushing's disease (Buchfelder *et al.* 1993). The number of significant ACTH pulses after hypophysectomy, however, was identified as a risk for recurrence of hyperadrenocorticism. Consequently, pulsatile ACTH secretion at 8 weeks after hypophysectomy is more likely to reflect the presence of residual adenomatous than unaffected corticotrophic cells in the pituitary fossa. In an earlier study, the CRH-stimulation test 8 weeks after hypophysectomy effectively identified dogs with residual disease but failed to differentiate between the cases that developed recurrence of hyperadrenocorticism and those that remained in remission (Meij *et al.* 1997a). Thus, the intrinsic ability of pulsatile hormone release is more informative for the prognosis than the response to CRH stimulation at 8 weeks after hypophysectomy. The slow growth rate of the corticotrophic adenoma may explain why hyperadrenocorticism recurs in 1·5–5 years. There was also a tendency that high postoperative AUC for GH was associated with a low risk of recurrence, which may be a reflection of normalized GH secretion of the residual somatotrophic cells after successful elimination of inhibition by high cortisol levels.

In conclusion, the results of the present study indicate that in PDH dogs, ACTH, cortisol,  $\alpha$ -MSH, and GH are secreted in a pulsatile fashion, and that hypophysectomy effectively reduces the secretion of ACTH and cortisol. The presence of ACTH pulses after hypophysectomy is associated with a higher risk of recurrence of hyperadrenocorticism.

## Acknowledgements

We are grateful for the technical assistance of Mr H G H van Engelen and Mrs J Wolfswinkel, and Dr G Voorhout for pituitary imaging and Dr W M Lee for data-typing. The critical reading of Prof. Dr A Rijnberk is highly appreciated. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Received 1 February 2006

Received in final form 29 May 2006

Accepted 6 June 2006