Residual pituitary function after transsphenoidal hypophysectomy in dogs with pituitary-dependent hyperadrenocorticism

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Abstract

Pituitary function was assessed before and after transsphenoidal hypophysectomy in 39 dogs with pituitary-dependent hyperadrenocorticism (PDH). Anterior pituitary function was investigated using combined administration of four hypophysiotropic releasing hormones (corticotropin-releasing hormone (CRH), GHRH, GnRH, and TRH) with measurements of ACTH, cortisol, GH, LH, prolactin (PRL), and TSH. Pars intermedia function was assessed by measurements of basal plasma α-MSH concentrations and adrenocortical function by baseline urinary corticoid/creatinine ratios.

At eight weeks after hypophysectomy basal plasma ACTH, cortisol, GH, LH, PRL, and TSH concentrations were significantly lower than before surgery. In seven dogs with elevated α-MSH concentrations, the values returned to the normal level after surgery. In the combined anterior pituitary function test there were no plasma GH, LH, PRL, and TSH responses to stimulation, whereas plasma ACTH and cortisol responses were small but significant. Remission of hyperadrenocorticism was obtained in 35 dogs and recurrences occurred in 3 of these within 16 months postoperatively. At 8 weeks after hypophysectomy, these 3 dogs were not discernible, with respect to residual pituitary and adrenocortical function, from the 32 dogs with persisting remission. Urinary corticoid/creatinine ratios in the latter group of dogs did not increase during 22 months after hypophysectomy.

In contrast to the presurgical findings, at 8 weeks after hypophysectomy there were significant positive correlations between baseline urinary corticoid/creatinine ratios and basal levels and responses for ACTH, indicating return to normal function of the pituitary–adrenocortical axis.

It is concluded that among the adenohypophyseal cells present in the sella turcica after hypophysectomy, the corticotropes have a distinct behavior. Much more so than the other cell types, the unaffected corticotropes tend to remain functional, or a repressed reserve fraction of corticotropes may become functional. This may be due to the removal of the hypothalamic influence of a postulated corticotropin-release inhibiting factor or a diminished inhibitory influence of a postulated paracrine factor. The corticotropes may maintain normocorticism, but may also lead to mild recurrence after relatively long periods of remission.

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Introduction

Since the late 1980s there has been consensus with regard to the therapy of Cushing’s disease. Transsphenoidal pituitary microsurgery is considered the primary treatment (Melby 1988, Thorner et al. 1992), despite a rate of recurrence of 20–30% (McCance et al. 1993, Sonino et al. 1996). In order to minimize the consequences of recurrences, parameters have been identified that might predict the risk of recurrence at an early stage. Adrenocorticotropin hormone (ACTH) hyperresponsiveness to an early postoperative stimulation with corticotropin-releasing hormone (CRH) seems to be a valuable criterion of recurrence (Vignati et al. 1994), although early postoperative measurements of plasma and/or urinary cortisol may safely replace tests using supra-pituitary stimulants (Pieters et al. 1989, de Lange & Sluiter 1993, Toms et al. 1993, Trainer et al. 1993, McCance et al. 1996).

In the debate over possible ways to decrease the number of recurrences and over the early detection of surgical failure, corticotropin cell hyperplasia has played a confounding role. It was presumed to be due to excessive production of hypothalamic CRH but in recent years it has been demonstrated that CRH concentrations in cerebrospinal fluid of both humans (Kling et al. 1991) and dogs (van Wijk et al. 1992) with pituitary-dependent hyperadrenocorticism (PDH) are lower than in healthy individuals. Moreover, there is now increasing evidence that the disease originates at the pituitary level by clonal expansion of a single aberrant cell (Pei & Melmed
and that corticotropin hyperplasia does not exist in Cushing’s disease (Trouillas et al. 1996).

Studies are therefore now directed at the biological basis of recurrence, i.e., at the proliferation potential of different pituitary cells and of adenoma cells (Laws et al. 1996). In this respect our recent reports on the development of a combined anterior pituitary test are of interest (Meij et al. 1996a,b). After hypophysectomy in healthy dogs, residual corticotropin cells were much more prone to respond in the combined anterior pituitary function test than were other types of anterior lobe cells (Meij et al. 1997a).

Under the assumption that this distinct behaviour of remnant corticotropin cells might play a role in the development of recurrent hyperadrenocorticism, we have now characterized pituitary function before and after hypophysectomy in dogs with PDH, a condition which has many similarities with Cushing's disease in humans (Kemppainen & Peterson 1994).

Materials and Methods

Animals and treatment

The group of dogs with PDH (n=39; median age 10 years; range 6–14 years) comprised 22 females (14 ovariohysterectomized) and 17 males (5 castrated). The diagnosis of hyperadrenocorticism was based upon the characteristic signs and symptoms, such as polyuria, skin atrophy, and increased abdominal size (Rijnberk et al. 1968), and upon elevated urinary corticoid/creatinine ratios in two consecutive morning urine samples (Stolp et al. 1983, Rijnberk et al. 1988a). Immediately after collection of the second urine sample, the animals received three oral doses of 0.1 mg dexamethasone/kg body weight at 8-h intervals. The next morning, a third urine sample was collected. When the ratio in the third sample was less than 50% of the mean in the first two samples, the dog was categorized as being responsive to dexamethasone suppression and PDH was diagnosed. In eight dogs in which there was less than 50% suppression of the corticoid/creatinine ratio, i.e., the dexamethasone-resistant animals, the diagnosis of PDH was secured by at least five measurements of basal plasma ACTH (Rijnberk et al. 1987), visualization of the adrenals by ultrasonography (Voorhout et al. 1990), and imaging of the pituitary gland by computed tomography (Voorhout 1990, Meij et al. 1997b). In all 39 dogs the baseline urinary corticoid/creatinine ratios exceeded the upper limit of the reference range (10 × 10−6) and ranged from 11 to 275 × 10−6 (median 56 × 10−6). In all 39 dogs plasma ACTH values (≥ 50 ng/l) were consistent with PDH and ranged from 51.0 to 606.7 ng/l (median 159.3 ng/l).

Transphenoidal hypophysectomy was performed according to a microsurgical technique described previously (Meij et al. 1997b). Immediately following removal of the pituitary gland, treatment was started with 1 mg hydrocortisone (Solu-Cortef; Upjohn, Ede, The Netherlands)/kg every 6 h (i.v.), and 0.01% desmopressin (Minrin; Ferring, Hoofddorp, The Netherlands), 1 drop every 8 h in the conjunctival sac. Drinking was allowed as soon as the dog was awake. When the dog had resumed drinking and eating, oral substitution therapy was started by administering 2 mg cortisone acetate (Cortisoni acetas; Genfarma, Maarssen, The Netherlands)/kg every 12 h and 10 µg thyroxine (l-thyroxine; Aesculaap, Boxtel, The Netherlands)/kg every 12 h, and the animals were discharged from the clinic. At home the dose of cortisone acetate was gradually lowered over a period of 4 weeks to 0.25–0.5 mg/kg every 12 h. Desmopressin was discontinued after 2–4 weeks. Daily water intake was measured by the owner for at least 4 weeks after surgery.

Stimulation test

After an overnight fast, the test was performed between 0800 and 1200 h. Anterior pituitary function was investigated before and at a median period of 8 weeks (range 6–18 weeks) after surgery by rapid sequential i.v. administration of four hypothalamic releasing hormones, as described previously (Meij et al. 1996a,b). Ovine CRH (Peninsula Laboratories, Belmont, CA, USA) and human growth hormone-releasing hormone (hGHRH; Peninsula Laboratories) were both stored frozen at −25 °C, and thawed at room temperature immediately before use. The gonadotropin-releasing hormone (GnRH) analogue, gonadorelin (Fertagyl; Intervet, Boxmeer, The Netherlands) and thyrotropin-releasing hormone (TRH; Hoffman-La Roche, Basel, Switzerland) were stored at 4 °C.

An i.v. catheter was placed in the cephalic vein of each dog to facilitate rapid sequential injections. The four releasing hormones were injected i.v. within 30 s, immediately following the collection of the zero blood sample from the jugular vein. The releasing hormones were injected in the following order and doses: 1 µg CRH/kg, 1 µg GHRH/kg, 10 µg GnRH/kg, and 10 µg TRH/kg. The clock for blood sampling times was started immediately after the administration of the last releasing hormone. Blood samples were collected by jugular vein puncture at −30, −15, 5, 10, 20, 30, 45, 60, 90, and 120 min (−15 to 45 min for plasma thyrotropin (TSH)) after injection and were placed in ice-chilled EDTA-coated plastic tubes. The samples were centrifuged at 4 °C for 10 min and plasma was stored at −25 °C until assayed for cortisol, ACTH, growth hormone (GH), luteinizing hormone (LH), prolactin (PRL), and TSH. Before and after surgery pars intermedia function was assessed by measurements of plasma α-melanotropin (α-MSH) in the −30, −15, and 0 min samples of the anterior pituitary function test.

Measurements of post-surgical corticoid/creatinine ratios in two consecutive morning urine samples were performed just prior to the anterior pituitary function test.
and at variable intervals thereafter. Each urine sample after surgery was collected after oral cortisone had been omitted for 24 h. Remission of PDH was defined as full remission of signs of hyperadrenocorticism and urinary corticoid/creatinine ratios <10 × 10⁻⁶.

**Hormone determination**

Adrenocorticotropic hormone was measured by RIA without extraction according to methods described previously (Arts et al., 1985, Rijnberk et al. 1988b). Antiserum was obtained from IgG Corporation (Nashville, TN, USA). The tracer was purchased from International CIS (St Quentin-Yvelines, France) and the standard was obtained from the National Institutes of Health (Bethesda, MD, USA). The intra-assay and interassay coefficients of variation were 8% and 12% respectively, and the detection limit was 10 ng/l.

Cortisol was measured by RIA (Rijnberk et al. 1988a,b). The detection limit for cortisol was 1 nmol/l and the intra-assay and interassay coefficients of variation were 6% and 8% respectively.

Growth hormone was measured in an homologous RIA as described previously (Eigenmann & Eigenmann, 1981). The intra- and interassay coefficients of variation were 3.8% and 7.2% respectively, and the sensitivity of the assay was 0.4 µg/l plasma. The degree of cross-reaction of canine prolactin was 2%.

Plasma LH was measured in an heterologous RIA (Nett et al. 1975). A rabbit antiserum raised against ovine LH (CSU-204), radiiodinated NIAMDD-bLH-4, and canine pituitary standard LER 1685-1 were used in this assay. The detection limit for LH was 0.31 µg/l and the intra-assay and interassay coefficients of variation were 2.3% and 10.5% respectively.

Plasma PRL was measured in an heterologous RIA (Stolp et al. 1986). The detection limit was 0.8 µg/l plasma and the intra-assay and interassay coefficients of variation were 3.5% and 11.8% respectively. The degree of cross-reaction of canine GH was 0.08%.

Plasma TSH was measured by immunoradiometric assay (IRMA) using a commercially available kit (Diagnostic Products Company, Los Angeles, CA, USA) (Williams et al. 1996). The sensitivity of the assay was 0.03 µg TSH/l. The intra- and interassay variation coefficients were 2.4% and 3.9% respectively, at mean TSH concentrations of 4.6 and 3.6 µg/l. There was negligible cross-reactivity of canine LH or follicle-stimulating hormone in the assay for canine TSH.

Plasma α-MSH was measured by RIA using antiserum to synthetic human α-MSH (Rijnberk et al. 1988b). This antiserum reacted equally with α-MSH and desacetyl-α-MSH. Synthetic human α-MSH was used as a standard. The detection limit was 10 ng/l and the intra-assay and interassay coefficients of variation were 10% and 23% respectively. A plasma α-MSH concentration above 60 ng/l was considered to be elevated (Rijnberk et al. 1988b).

**Statistical analysis**

Results are presented as means ± s.e.m. Mean basal levels were calculated from the −30, −15, and 0 min values (ACTH, cortisol, GH, LH, PRL, and α-MSH) and the −15 and 0 min values (TSH) in the stimulation test. In the stimulation tests increments of plasma concentrations were calculated as the difference between peak levels and mean basal levels. The areas under the curve (AUC) of the hormone concentrations in the stimulation tests were calculated by the trapezoidal method after subtraction of the mean basal level. Differences between pre-operative and postoperative basal levels, increments, and AUC for all dogs (n=39) were analyzed by Student’s t-test for paired samples. Differences in hormone variables between the dogs with urinary corticoid/creatinine ratios ≤ 5 × 10⁻⁶ (n=35) and dogs with ratios > 10 × 10⁻⁶ (n=4) at 8 weeks after surgery were analyzed by Student’s t-test for independent samples. Differences in basal levels and responses for LH between noncastrated dogs (n=20) and castrated dogs (n=19) were analyzed by Student’s t-test for independent samples. Pearson’s correlation coefficients (two-tailed) were calculated between urinary corticoid/creatinine ratios and basal values and responses after stimulation for both plasma ACTH and cortisol. Correlations were also calculated between the corticotropic (ACTH and cortisol) variables and other hormone (GH, LH, PRL, TSH, and α-MSH) variables. Differences in median urinary corticoid/creatinine ratios at 8 weeks, and at 16 and 22 months after hypophysectomy were analyzed by the nonparametric Wilcoxon matched-pairs signed-ranks test. P<0.05 was considered significant.

**Results**

Basal plasma levels for ACTH, cortisol, GH, LH, PRL, and TSH were significantly lower at 8 weeks after hypophysectomy than before surgery (Table 1, Fig. 1). No side effects were observed after the combined administration of releasing hormones in the dogs with PDH, either before or after hypophysectomy. Before hypophysectomy, hypophysiotropic stimulation caused prompt increases in plasma ACTH, cortisol, GH, LH, PRL, and TSH in all 39 dogs (Fig. 1). The peak plasma ACTH, cortisol, GH, LH, PRL, and TSH concentrations were (mean ± s.e.m.): ACTH, 469 ± 9 ± 64·2 nmol/l at 20 min (Fig. 1a); cortisol, 808·1 ± 64·2 mmol/l at 20 min (Fig. 1b); GH, 5·0 ± 1·0 ± 0·1 µg/l at 20 min (Fig. 1c); LH, 41·0 ± 4·3 µg/l at 10 min (Fig. 1d); PRL, 124·4 ± 20·4 µg/l at 5 min (Fig. 1e); TSH, 0·59 ± 0·05 µg/l at 20 min (Fig. 1f). At 8 weeks after hypophysectomy there were no plasma GH,
LH, PRL, and TSH responses to stimulation (increments and AUC approximately zero), whereas plasma ACTH and cortisol responses were small but significant (Table 1, Fig. 1). After hypophysectomy the maximum plasma ACTH, cortisol, GH, LH, PRL, and TSH concentrations, increments and area under the curve (AUC) after combined administration of four hypothalamic releasing hormones (CRH, GHRH, GnRH, and TRH) in 39 dogs (20 non-castrated dogs and 19 castrated dogs) with pituitary-dependent hyperadrenocorticism, before and at 8 weeks after transsphenoidal hypophysectomy (HX). Values represent the means ± S.E.M. (Table 1).

<table>
<thead>
<tr>
<th>Basal</th>
<th>Increment</th>
<th>AUC (0–120 min)</th>
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<tr>
<td></td>
<td>Before HX</td>
<td>After HX</td>
</tr>
<tr>
<td>ACTH (ng/l)</td>
<td>1747 ± 181</td>
<td>679 ± 84³</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>3425 ± 374</td>
<td>1184 ± 14²</td>
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<tr>
<td>GH (µg/l)</td>
<td>1.1 ± 0.1</td>
<td>0.8 ± 0.0³</td>
</tr>
<tr>
<td>LH (µg/l)</td>
<td>5.6 ± 0.8</td>
<td>3.6 ± 0.5³</td>
</tr>
<tr>
<td>Non-castrated (n=20)</td>
<td>23.8 ± 3.8³</td>
<td>3.0 ± 0.2³</td>
</tr>
<tr>
<td>Castrated (n=19)</td>
<td>12.4 ± 0.7</td>
<td>5.5 ± 0.3³</td>
</tr>
<tr>
<td>PRL (µg/l)</td>
<td>0.22 ± 0.03</td>
<td>0.03 ± 0.00³</td>
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<tr>
<td>TSH (µg/l)</td>
<td>0.44 ± 0.07</td>
<td>0.02 ± 0.00³</td>
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³P<0.05 (Student’s t-test for paired samples) compared with preoperative values; ³P<0.05 (Student’s t-test for independent samples) compared with values in non-castrated dogs; AUC (0–45 min).

In three dogs (nos. 13, 21, and 25) remission of hyperadrenocorticism was initially achieved with corticoid/creatinine ratios of 0.3, 3.5, and 0.6 × 10⁻⁶ respectively, at 8 weeks after hypophysectomy. However, at respectively 11, 7, and 16 months postoperatively, signs of hyperadrenocorticism recurred and the ratios had increased to values >10 × 10⁻⁶ (Table 1, Fig. 1e), there was a significant correlation (n=35) between ACTH and PRL basal levels (r=0.48, P<0.005).

In the latter two dogs (nos. 21 and 25) contrast-enhanced CT revealed no remnant pituitary tissue. At 8 weeks after hypophysectomy, all dogs were free of signs of hyperadrenocorticism, i.e. 12 months postoperatively. Prior to surgery, at 2 and 12 months after hypophysectomy, basal plasma ACTH concentrations in dog no. 13 were
Figure 1 Plasma ACTH (a), cortisol (b), GH (c), LH (d), PRL (e), and TSH (f) responses (mean ± S.E.M.) in 39 dogs (LH (d): 20 noncastrated (●, ○) and 19 castrated (■, □) dogs) with pituitary-dependent hyperadrenocorticism after the rapid (30 s) i.v. injection (arrow) of a combination of four hypothalamic releasing hormones, before (●, ■) and at 8 weeks after (○, □) transsphenoidal hypophysectomy. The releasing hormones were injected in the following order and doses: 1 µg CRH/kg body weight (bw), 1 µg GHRH/kg bw, 10 µg GnRH/kg bw, and 10 µg TRH/kg bw. Basal levels and increments in plasma ACTH, cortisol, GH, LH, PRL and TSH were significantly lower after hypophysectomy (Student's t-test, P<0.05).
164.3 ng/l, 17.3 ng/l, and 153.7 ng/l respectively; the corresponding plasma ACTH increments at the same times were 98.7 ng/l, 37.7 ng/l, and 863.3 ng/l respectively. CT repeated in dog no. 13 at 1 month after recurrence, i.e. 12 months postoperatively, showed that the localized areas of contrast enhancement in the pituitary fossa had increased in size compared with the CT image at 8 weeks after hypophysectomy.

In 32 dogs, hyperadrenocorticism remained in remission for a median follow-up period of 22 months (range 2 to 42 months) and urinary corticoid/creatinine ratios ranged from 0.1 to 5.9 × 10⁻⁶ (median 2.0 × 10⁻⁶). At that time urinary corticoid/creatinine ratios were ≤ 1.0 × 10⁻⁶ in 9 dogs and > 1 and ≤ 5.0 × 10⁻⁶ in 22 dogs, whereas in one dog the urinary corticoid/creatinine ratio varied between 5 and 10.0 × 10⁻⁶. In the dogs in which hyperadrenocorticism remained in remission there were no differences in median urinary corticoid/creatinine ratios at 8 weeks (1.5 × 10⁻⁶, 35 dogs), 16 months (2.0 × 10⁻⁶, 33 dogs), and 22 months (2.0 × 10⁻⁶, 32 dogs) after hypophysectomy (Wilcoxon matched-pairs signed-ranks test, P < 0.05). In 2 dogs (nos. 1 and 5) that died of unrelated causes while in remission at respectively 5 and 2 months postoperatively, microscopic examination of the sella revealed islets of nontumorous adenohypophyseal tissue embedded in fibrous tissue.

Prior to surgery there were seven dogs with elevated basal plasma α-MSH values (352.7 ± 105.4 ng/l). In the other 32 dogs the values (21.8 ± 2.1 ng/l) were not elevated (Fig. 3). In the group of seven dogs plasma α-MSH concentrations were significantly lower after hypophysectomy (19.5 ± 2.2 ng/l) compared with preoperative values, and not significantly different from plasma α-MSH concentrations in the group of 32 dogs, either before or after hypophysectomy (20.3 ± 2.9 ng/l) (Fig. 3).

**Discussion**

Complete hypophysectomy should result in very low urinary corticoid/creatinine ratios. In the present study we defined remission by the complete disappearance of signs of hyperadrenocorticism together with urinary corticoid/creatinine ratios below the upper limit of the reference range, that is < 10 × 10⁻⁶ (Rijnberk et al. 1988a). Measurable urinary corticoid/creatinine ratios < 5 × 10⁻⁶ at 8 weeks after surgery in 35 dogs, although the dogs were in remission, suggested that there was some residual function of the pituitary–adrenocortical axis. This was confirmed by the ACTH and cortisol responses in the combined anterior pituitary function test in almost all dogs.

In the combined anterior pituitary function test performed at 8 weeks after hypophysectomy, there were
no plasma GH, LH, PRL, or TSH responses to hypo-
physiotropic stimulation, whereas plasma ACTH and
cortisol responses were small but significant. The cortico-
tropic response to stimulation after hypophysectomy is
highly suggestive of residual function by small islets of
pituitary cells in the sella turcica containing corticotrophic
cells, as observed in a previous study in healthy beagle dogs
(Meij et al. 1997a). After surgery, in contrast to before,
urinary corticoid/creatinine ratios and basal and stimulated
plasma ACTH levels in the hyperadrenocorticol dogs
were positively correlated, indicating restoration of the
normal regulatory mechanisms.

Thus, we have here the intriguing observation in both
healthy dogs and dogs with PDH that of the few remnant
cells in the sella, only the corticotrophic cells tend to be
functional. As it was found not only in the dogs with PDH
but also in the healthy dogs, the phenomenon cannot be
ascribed to persistent tumor. In addition, after hypo-
physectomy in the dogs with PDH, the restoration of
normal regulation, the low urinary corticoid/creatinine
ratios, the long-term remission, and the finding of only
normal pituitary tissue in the sella of two dogs in remission
that died of unrelated disease, argue against adenoma
tissue being the cause of the ACTH release after hypo-
physectomy. Three possible explanations will be presented
for this remnant nontumorous ACTH secretion follow-
ing hypophysectomy. One is related to the decreased
inhibitory effect of glucocorticoids. The second and the
third deal with the possibility that the sellar fragments are
devoid of either hypophysiotropic inhibitory influences or
inhibitory paracrine influences.

First, structural changes in pituitary corticotropes
are known to correlate with changes in the physiological
state of the animal (Childs 1992). For example, in rats
both the number and the structure of corticotropes change
after adrenalectomy or chronic stimulation with CRH.
Secretagogues like CRH or arginine vasopressin (AVP)
increase the percentages of corticotropes that bind CRH
and store ACTH which has led to the idea of the existence
of reserve cells that may be sensitive to certain levels or
types of stimuli (Childs 1992). Thus, if hypophysectomy
has not been complete and some suppressed but otherwise
unaffected corticotropes remain in the sella turcica, their
function is first restored by cessation of the glucocorticoid
excess. The low cortisol levels after hypophysectomy will
also stimulate release of hypophysiotropic hormones such
as CRH and AVP and consequently the corticotropes may
increase in number. The striking similarity with respect
to the residual corticotropic response following hypo-
physiotropic stimulation in normal dogs (Meij et al. 1997a)
and dogs with PDH (this study) suggests that this
mechanism may cause remaining normal corticotropes to
become functional again and subsequently increase in
number by chronic stimulation.

A second or concurrent option is that residual cortico-
tropic response may be due to the removal of hypo-
thalamic inhibitory input to the residual cells. The existence of a hypothalamic corticotropin-release inhibiting
factor has long been proposed on the basis of hypothalamo–pituitary disconnection studies in several
species, including the dog (Egdahl 1960, Halász et al. 1967,
Engler et al. 1990, Redei et al. 1995). These studies have
consistently reported elevated basal levels of ACTH and
glucocorticoids, and increases in anterior pituitary pro-
opiomelanocortin (POMC) mRNA levels, suggesting that
disconnection removes inhibitory influences, presumably
of hypothalamic origin, from the corticotropic cell.

Thirdly, there is increasing evidence that local com-
munication among cells of the anterior pituitary plays an
important role in the regulation of ACTH secretion
(Schwartz et al. 1989, Schwartz 1990). Further investiga-
tion has led to the conclusion that paracrine communi-
cation among distinct fractions of corticotropes of the
anterior pituitary regulates ACTH secretion (Jia et al.
1992). It was postulated that a paracrine factor profoundly
inhibits CRH-stimulated ACTH secretion from a re-
pressed fraction of corticotropes, a mechanism which
functions to hold corticotropes in reserve (Jia et al. 1992).
Thus it can be hypothesized that, as has been found in
healthy dogs (Meij et al. 1997a), after hypophysectomy
in dogs with PDH, a critical intercell distance may be
exceeded between dislodged pituitary cells in the sella
turcica. The absence of the inhibitory effect of the
postulated paracrine factor would allow the corticotropes
among the pituitary cells and the reserve cells to become
responsive to CRH. The latter mechanism may contribute
to the gradual development of higher cortisol levels and a
residual response to hypophysiotropic stimulation after
hypophysectomy.

In the dog the dorsoventral axis of the hypophysis is
orientated in a horizontal plane (Hullinger 1993). Using
the ventral transphenoidal approach in the dog (Meij et al.
1997b), the centroventral portion of the pars distalis
is the first part of the hypophysis to be encountered.
This portion and the rostral portion of the pars distalis contain
high concentrations of corticotropic and lactotrophic
cells (El Etreby & Fath El Bab 1977, El Etreby & Dubois 1980).
From a surgical anatomical viewpoint the rostral portion
of the pars distalis is considered to be the region which is
most difficult to remove, which seems to be consistent
with our finding that after surgery basal ACTH levels
were correlated with basal PRL values. Corticotropic cells
are much less frequent in the pars tuberalis than in the pars
distalis of the adenohypophysis in the dog (El Etreby &
Dubois 1980, Halmi & Krieger 1983) and the presence of
remnants of pars tuberalis after hypophysectomy would
therefore not provide an adequate explanation for a
significant residual corticotropic response. Moreover, it
was shown previously that no remnants of pars tuberalis
were observed on the ventral hypothalamus following
hypophysectomy in normal dogs (Meij et al. 1997a,b). The
origin of the corticotropic cells responsible for the residual
corticotropic response is therefore most likely the rostral portion of the pars distalis. The pars intermedia of the dog is cytologically heterogeneous. The predominant A cells are typical pars intermedia cells: they stain immunocytochemically for α-MSH and less strongly for ACTH. The cells of the pars distalis, i.e. they stain intensely for ACTH but not at all for α-MSH (Halmi & Krieger 1983). The pars intermedia in the dog is under tonic dopaminergic inhibition (Zerbe et al. 1993). Thus, corticotropes and melanotropes in isolated pars intermedia fragments in the sella after hypophysec tomomy are devoid of the dopaminergic melanotropes in isolated pars intermedia fragments in the sella after hypophysectomy are devoid of the dopaminergic inhibitory influences, and this will favor secretory activity.

The predominant A cells are typical pars intermedia cells: they stain immunocytochemically for ACTH and α-MSH, but not at all for α-MSH (Zerbe et al. 1993). Thus, corticotropes and melanotropes in isolated pars intermedia fragments in the sella after hypophysectomy are devoid of the dopaminergic inhibitory influences, and this will favor secretory activity.

Canine PDH is usually caused by pars distalis adenomas but the excess of POMC-derived peptides may also originate from neoplastic transformation of the pars intermedia, resulting in high α-MSH levels. The CSU-204 antiserum and Dr B E Belshaw is highly appreciated.

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