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Corticotroph adenoma in the dog: Pathogenesis and new therapeutic possibilities

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ABSTRACT

The corticotrophinoma, causing pituitary dependent hypercortisolism, represents the highest percentage of pituitary tumours in the dog. The mechanism by which it develops is currently unknown and two theories are postulated: the hypothalamic and the monoclonal. It is not clear either what factors are involved in the tumour genesis; nevertheless, firm candidates are the Rb1 gene, proteins p27, p21 and p16, as are also defects in the glucocorticoid receptor and Nur77/Nurr1. The role of BMPs remains to be evaluated in greater depth. Although at present the chosen treatment in human is surgical, there are various pharmacological treatments already in use that have favourable results and others, still under research, also showing promising results.

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1. Introduction

The corticotroph adenoma or corticotrophinoma, causing Cushing's disease (CD) or Pituitary dependent hypercortisolism (PDH) in humans represents 6% of pituitary Tumours (Gsponer et al., 1999; Chanson and Salenave, 2004). On the other hand, this pituitary tumour is observed frequently in the dog, with other types of pituitary neoplasms being rare (Fracassi et al., 2007).

It has a high morbidity rate, both in human and dogs, because of the adverse effects of the excess of cortisol due to adrenal hyperstimulation and the effect the tumour mass has on the brain (Orth and Kovacs, 1998; Reichlin, 1998). To understand the pathogenesis and further development of this tumour, one has to take into account the embryological origin of the corticotroph area. Like the rest of the adenohypophysis, it originates in the Rathke pouch coming from the lingual ectoderm, differing from the other adenohypophysis cells because of the expression of other transcription factors, particularly Ptx-1 and Tpit, LIF (leukaemia inhibiting factor) and the corticotroph releasing factor (CRH) (Akita et al., 1995; Dasen et al., 1999; Lamolet et al., 2004; Lanctôt et al., 1999; Ooi et al., 2004; Savage et al., 2003; Suh et al., 2002; Treier et al., 1998). In the dog, corticotroph cells are not only found in the anterior lobe of the pituitary but also in the pars intermedia, with the latter being innervated by dopamine axons which regulate the synthesis of pro-opiomelanocortin (POMC) and its peptide derivates. The increase in dopaminergic tone results in the inhibition of the synthesis of this macromolecule in the pars intermedia and in the anterior lobe (Tanaka, 2003; Colao et al., 2000). Biosynthesis and secretion of adrenocorticotrophin (ACTH) is co-ordinately controlled by different transcription factors at the level of the POMC gene promoter, such as CRH, vasopresin (VP) and LIF. CRH induces the expression of c-FOS and the nerve growth factor induced gene-B (NGFI-B or Nurr77/Nurr1).It also induces Nurr77/Nurr1 transcriptional activity (necessary for POMC synthesis) and that of AP-1 and CREB (Philips et al., 1997; Savage et al., 2003; Seaholtz, 2000). Inhibition is mainly determined by glucocorticoids (GC) through the glucocorticoid receptor (GR). Martens et al. (2005) propose that GC antagonize Nur77 activity, thus inhibiting POMC synthesis. There would seem to be other inhibiting factors interacting (Engler et al., 1999).

Nudi et al. (2005) suggested that the bone morphogenic protein-4 (BMP4) acts inhibiting POMC gene expression through Small mothers against decapentaplegic homolog (SMADs), particularly SMAD1, interfering with Pitx1 and Tpit activity, as well as taking part in the embryologic development of the hypophysis (Ooi et al., 2004; Savage et al., 2003). It is thought that BMP-4 has an autocrine and paracrine role in corticotrophs and maintains the balance between the different pituitary cell lines (Nudi et al., 2005). Embryologic and functional characteristics of corticotroph cells have been the basis for explaining the reasons corticotrophinomas appear. Regarding treatment, the first choice in humans is pituitary surgery, with radiotherapy being proposed for children and adolescents (Boscaro et al., 2001; Brada, 1993). In veterinary medicine, surgery has been largely developed by the group at

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Utrecht University, obtaining good results (post surgical survival of 3 years in 79% of the cases), with a low recurrence index (15%) similar to that in human medicine (Mampalam et al., 1988; Simmons et al., 2001). However both surgery and subsequent medical treatment are complex (Meij, 2001; Meij et al., 2002; Hanson et al., 2005, 2007) therefore unfortunately they are not indicated in the majority of hospital-university establishments of other countries. Post surgical morbidity in intensive care and at long term must also be considered (Meij et al., (1997,2002)) as well as those cases in which surgery is contraindicated. In cases where radiotherapy is indicated, the risks are the collateral effects on the area in the vicinity of the tumour (Boscaro et al., 2001).

Different drugs have been proposed for medically treating CD in dog and human with varying success. Dopaminergic drugs (bromoergocriptin and selegylin) and serotoninergic drugs have been used to try to control ACTH secretion and tumour growth (Braddock et al., 2004; Petrossians et al., 2000; Reusch et al., 1999). Therapies with drugs acting on the adrenal gland are indicated in certain cases (such as prior to surgery, no tumour being found, surgical contraindication). These drugs act by inhibiting cortisol biosynthesis, for example ketoconazole, trilostane and aminoglutetimide (Barker et al., 2005; Castillo et al., 1996; Engelhardt and Weber, 1994; Sieber-Ruckstuhl et al., 2006); or by antagonizing glucocorticoid receptors, for example mefepristone (Agarwal, 1996) or by provoking necrosis of the adrenal cortex, for example Op'-DDD (Kintzer and Peterson, 1991; den Hertog et al., 1999). The aim of this manuscript is to explain the theories and causes proposed for the appearance and development of the corticotrophinoma and new therapeutic alternatives that can be used in the treatment of this tumour.

2. Corticotrophinoma pathogenesis: hypothalamus vs. hypophysis

Two theories have been put forward to explain the development of the corticotrophinoma. One is the *hypothalamic theory*. On the one hand, the hypothalamus would exert excess stimulation on the corticotroph area through a greater secretion of CRH and VP (Dahia and Grossman, 1999). Also, defects in GR would lead to a greater stimulation of the corticotroph cells due to a lower inhibitory action of cortisol (Lamberts, 2002) and therefore an increase in POMC synthesis and in CRH. On the other hand, dopaminergic neurodegeneration in aged individuals (Bruyette, 1995; Hereñú et al., 2006a,b) or a decreased expression of the D2 dopaminergic receptor in the corticotroph cells could result in a lower inhibition on the corticotroph area (Colao et al., 2000; de Bruin et al., 2008; Pivonello et al., 2004) thus producing hyperplasia. The adenoma would then evolve because of a somatic mutation of a hyperplasic cell. This line of thought is reinforced by the recurrence of the tumour or in certain cases when the tumour is not found. Supporting this hypothesis, individuals with chronic stress where there is a greater activation of the hypothalamus-hypophysis-adrenal axis (HHA), also show corticotroph hyperplasia (Otte et al., 2005; Sonino et al., 1993). Regarding resistance to GC, Karl et al. (1996a,b) and Lamberts (2002) describe a mutation in the gene that encodes the GR, with a reduction in the sites of DNA binding while maintaining affinity for cortisol. This de novo mutation in the germinal line provokes a general resistance to GC that precedes the formation of the corticotrophinoma. They describe a loss of heterozigosity (LOH) in the GR locus in tumours secreting ACTH. According to Lamberts (2002), there would be a combined effect between chronic stimulation of the corticotroph by increase of CRH or VP and the loss of negative feed-back to decrease the sensitivity to cortisol. Recent studies by Teshima et al. (2009a,b), using trilostane to decrease cortisol leading to tumour growth as a consequence of the reduction of the negative feedback (2009a) and their studies on tumoral corticotroph cells in dogs (2009b) reinforce what Lamberts (2002) has proposed. These events would first lead to corticotroph hyperplasia and then there would be at least some somatic mutation in the proteins that control the cell cycle, leading to tumour development (Dahia and Grossman, 1999).

Against the hypothalamic hypothesis, it is argued that no hyperplasic area has been found surrounding the corticotrophinomas that have been removed, in addition to the recovery and non recurrence in 80% of the cases and, what is more important: the characterization of a monoclonal state in the majority of the adenomas studied (Dahia and Grossman, 1999; Levy and Lightman, 2003).

The second theory is the *monoclonal theory*. This proposes the pituitary as being the only origin of the adenoma, through the somatic mutation of a corticotroph cell developing a tumour clone. This mutation precedes the clone expansion of the tumour (Herman et al., 1990). This is currently the most accepted theory because most adenomas studied show this origin (Dahia and Grossman, 1999; Levy and Lightman, 2003). Still under discussion is which mutation provokes the appearance of the tumour, and where; as are the factors involved in its development. It is possible that in a few cases the monoclonal pattern is acquired later on in its development, after a prolonged phase of polyclonal growth in response to stimulating factors (Dahia and Grossman, 1999; Lamberts, 2002). In this case, a selection pressure would exist in favour of a certain cell clone, which would develop, annulling the rest of the clones (Clayton and Farrell, 2001; Levy and Lightman, 2003). Taking into account micro and macroadenomas, the existence of a variety of corticotrophinomas is suggested. Macroadenomas can display from a limited growth and benign behaviour, to an aggressive behaviour as in Nelson's case (Assa and Ezzat, 1998).

3. Proposed factors involved in the appearance of the corticotrophinoma

3.1. Proto-oncogenes

Studies carried out on c-Erb B2/neu, c-MYC and PKC, RET, c-FOS, c-MYB and RAS oncogenes have not shown abnormalities that would suggest their participation in the development of the corticotrophinoma (Dahia and Grossman, 1999). What has been found over-expressed is the tyrosine kinase c-MYC proto-oncogene (Evans et al., 2001). The pituitary tumour transforming gene (PTTG) which is expressed in proliferating cells (controlling chromatin separation) has been proposed in the development of various pituitary adenomas (Zhang et al., 1999; Melmed, 2003). A 50% increase in PTTG was found in only 1 corticotrophinoma of a series of various human pituitary tumours (Zhang et al., 1999). It is not yet clear whether over-expression of PTTG has any role in pituitary tumour formation and/or its subsequent growth (Heaney et al., 2002a; Levy and Lightman, 2003).

3.2. Suppressor tumour genes (proteins associated to the cell cycle)

Protein p53 (which blocks progression of the cell cycle as from G1) was inconsistently found by immunohistochemistry in the corticotrophinomas and in invasive pituitary adenomas (Buckley et al., 1995). Although there is no evidence of the existence of a mutation of this protein that would induce the appearance of the corticotrophinoma, it has been proposed that p53 would have a role in the aggressive behaviour of the tumour (Dahia and Grossman, 1999; Levy and Lightman, 2003). The family of cell cycle inhibitory proteins (p21, p27 and p16) as well as the retinoblastoma gene (Rb1, in its de-phosphorylated form which inhibits

progression of phase G1 to S) have been studied in corticotrophinomas. Knock-out mice for Rb1 and p27 genes have been described to develop tumours in the pars intermedia (Jacks et al., 1999; Nakayama et al., 1996), indicating that both proteins play an important role in tumour pathogenesis. Decreased levels of p27 have been described in various corticotrophinomas, which suggests that post transcriptional mechanisms of inactivation could be possible (Levy and Lightman, 2003; Lidhar et al., 1999).

3.3. Receptor modulation

It has been observed that receptors for CRH (CRHR) and VP (subtype V3, VPR3) are over expressed in corticotrophinomas (Dahia and Grossman, 1999; Melmed, 2003; Zeugswetter et al., 2008). VPR3 was also found to be increased under certain stressful conditions (Sonino et al., 1993). While chronic stress is associated with a decrease in the number of CRHR the opposite occurs for VP3R, where the proliferative signal is increased (Thibonnier et al., 1997). This chronic stimulation of the corticotroph area would lead to or aid clonal growth of the corticotroph cell. CRHR are over expressed in corticotrophinomas; nevertheless, it is not clear whether this is provoked by a greater transcription of the receptors, reflecting an abnormal response from the tumour, or if it is due to a defective processing at the post transcriptional level (Levy and Lightman, 2003; Dieterich et al., 1998). LIF involvement in corticotrophinoma pathogenesis has not been established (Levy and Lightman, 2003). Nevertheless, Yano et al. (1998) described corticotroph hyperplasia and Cushing-like characteristics in transgenic mice as a consequence of the expression of this cytokine.

ACTH secreting tumour resistance to GC has been widely communicated (Karl et al., 1996a,b; Lamberts, 2002). As mentioned in the introduction, resistance to GC precedes the appearance of the corticotrophinoma owing to a dominant-negative mutation. Nevertheless, GR mutation is not a common event (Levy and Lightman, 2003). It is feasible that certain types of corticotrophinoma are associated with the defects described in the receptor, while these events are irrelevant in others (Lamberts, 2002; Levy and Lightman, 2003). Recently Teshima et al. (2009b) have suggested an alteration of GR in dogs with corticotrophinoma, affecting the expression of 11 beta hydroxysteroid dehydrogenase type-1 and type-2 enzymes (decreased and increased expression respectively). They conclude that these changes may have a profound role in the growth of canine corticotroph adenomas and may help to further understand the pathophysiology of dogs with CD.

Nur77/Nurr1, members of the family of receptors for steroid hormones, are the main intermediaries for CRH induced POMC synthesis, blocking the inhibitory signal of GC (Maira et al., 1999; Philips et al., 1997; Savage et al., 2003). CRH acts via calcium, mitogenic protein kinase A (MAPK) to induce and activate Nur77/Nurr1 (Kovalovsky et al., 2002), having a proliferative effect. Based on reports by Martens et al. (1997) and by Kovalovsky et al. (2002), over-expression of Nur77/Nurr1 could lead to GC resistance and/or cell proliferation.

3.4. Genes involved in the development and regulation of pituitary growth

Homebox genes: despite their role in the embryogenesis of the pituitary, and of the corticotroph in particular, "homebox genes Ptx" activity in tumour pathogenesis has not been defined (Dahia and Grossman, 1999; Levy and Lightman, 2003). Analyzing TPIT expression in corticotroph cells, Vallette-Kasic et al. (2003) found a greater expression of TPIT in tumour cells with respect to normal cells. These authors also demonstrated that this expression does not occur in other types of pituitary tumours. Nevertheless, in a recent study Hanson et al. (2008) concluded that mutations in the TPIT gene are unlikely to play a major role in the pathogenesis of canine corticotrophinomas.

3.5. Other factors involved in the genesis of corticotroph tumours

Sugawara et al. (1995) described the expression of the retinoid (RXR) and retinoic acid receptor (RAR) in the pituitary, where they intervene in cell differentiation and growth regulation (Zhou et al., 1999). According to Merino and Hurlé (2003) retinoic acid interacts with BMP's, both through the SMADs and MAPK via, having in this way a role in the corticotroph cell growth and in the expression of the POMC promoter gene. The MAPK via would be related to pro-apoptotic and anti-proliferative activity of retinoic acid observed in certain tumours (Chen et al., 2004; Lei and Thé, 2003; Merino and Hurlé, 2003). As has been recently described by Giacomini et al. (2006) defects in RXR/RAR or in BMP's signalling, would lead to the loss of the corticotroph cell regulation and could be a factor promoting the development of the ACTH producing adenoma. Peroxisome proliferator-activated receptor gamma (PPAR-γ) is expressed in corticotroph cells, both from normal pituitaries and from those with tumours in mice and humans (Heaney et al., 2003). PPAR-γ is restricted to and localized in cells that secrete ACTH in the pars distalis of the pituitary (it is not expressed in the pars intermedia of mice). PPAR-γ was abundantly expressed in all of 6 evaluated corticotrophinomas (Heaney et al., 2002b) (Fig. 1). PPAR-γ function in corticotroph cells (and in the pituitary in general) is as a transcription factor of ligands dependent on the synthesis process of mRNA, therefore regulating POMC synthesis stimulated by CRH and acting in the cell cycle regulating its mitogenic effect (Heaney et al., 2002b, 2003; Lovekamp-Swan and Chaffin, 2005).

4. Conventional and current therapies

Conventional and current therapies have been mentioned in the introduction. The ones that act on the pituitary are generally ineffective. In human patients with PDH, antiserotoninergic drugs, such as cyproheptadine, ritanserin and ketanserin haven't shown good results (Sonino et al., 2000; Arnaldi et al., 2007). In dogs, selegiline, a monoamine oxidase-B inhibitor, could not control the disease satisfactorily (Reusch et al., 1999; Braddock et al., 2004).

Drugs acting on the adrenal gland have been extensively studied and seem to be useful in the treatment of PDH in dogs (den Hertog et al., 1999; Ruckstuhl et al., 2002; Wenger et al., 2004; Barker et al., 2005; Reine, 2007; Sieber-Ruckstuhl et al., 2008). On the other hand, although they solve the hypercortisolism or its effects, and therefore the clinical signs, they do not resolve the cause: the corticotrophinoma. Furthermore regarding trilostane therapy, tumour growth can progress (Teshima et al., 2009a).

5. Future therapies for treating the corticotorphinoma

Three new therapies, which either have been or are currently under study, will now be described.

5.1. Tiazolindindiones (TZD)

Studies with TZD have been started keeping in mind the PPAR-γ expression previously described and the effects on cell regulation (Lei and Thé, 2003; Lovekamp-Swan and Chaffin, 2005). Heaney et al. (2002b) studied the effect of rosiglitazone (Ros) both *in vitro*, in cell culture with AtT20 tumour corticotrophs, and *in vivo,* in nude female mice (nu/nu); this being the first work

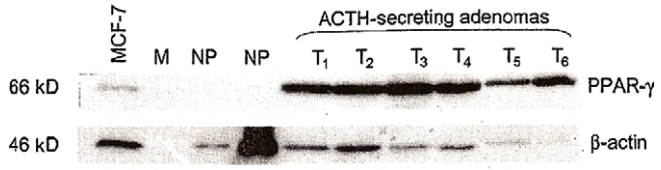

Fig. 1. PPAR-γ expression in the corticotrophinoma. Western-blot of 6 corticotrophinomas showing PPAR-γ expression (T1 to T6) versus 2 normal pituitary (NP). β-actin immunoblotting confirms the equivalent of the total protein loaded. (Taken from Heaney et al. (2002a,b). Permission obtained from Nature Medicine, Copyright 2002, Nature Publishing Group).

carried out on the use of Ros in PDH. The authors observed the arrest of the cell cycle in G0/G1 when administering Ros *in vitro* (preventing the phosphorylation of Rb1, they increase p21 and p27), the induction of corticotroph apoptosis (anti-apoptotic protein Bcl-2 decreases and pro-apoptotic Bax increases) and a decrease in the synthesis of POMC and ACTH. In cells treated with Ros, as the rate of cell proliferation decreases, a lower expression of the PTTG gene is also described. *In vivo* studies, carried out inoculating a group of five mice with AtT20, showed Ros preventing tumour development in four of the five treated mice while all controls showed tumour growth after 4 weeks. ACTH and cortisol concentration was significantly greater in controls when compared to the treated animals. To know if Ros blocked previously inoculated untreated tumours, they allowed tumour growth in five mice and then administered Ros (and compared results to controls without Ros). Although growth continued in both groups, growth was lower than that of controls in three out of five mice treated with Ros. PTTg mRNA expression decreased 80% with respect to the controls, and approximately 60% POMC mRNA expression decreased, confirming *in vitro* observations. ACTH concentration was 75% lower and corticosterone showed a 96% reduction in Ros treated mice with respect to the controls and, contrary to the controls, treated mice did not present clinical signs of the disease. Based on these findings, the authors conclude that TZDs show a potential use in patients with CD (Ambrosi et al., 2004). Similar results have been observed with pioglitazone (Suri and Weiss, 2005).

5.2. Retinoic acid (RA)

RA has been studied in various types of tumours (Lei and Thé, 2003; Páez-Pereda et al., 2000). It inhibits cell proliferation, growth and invasion and induces cell apoptosis and differentiation (Merino and Hurlé, 2003). Some of these effects are due to inhibition of AP-1 and of the orphan Nur77 receptor (Kang, 2000). Based on these effects of RA and on its activity in the corticotroph cell (see introduction) Páez-Pareda et al. (2001) examined the effects RA would have on the ACTH secreting tumour cell. In AtT-20 cell cultures, RA only inhibits transcriptional activity of AP-1 and Nur77/Nurr1, thus inhibiting POMC and ACTH synthesis. In human corticotroph cells, obtained from ACTH secreting tumours and from different cell populations of normal pituitaries, ACTH biosynthesis was only inhibited in tumour corticotroph cells, while normal cells were unaffected. Neither was the synthesis of prolactin and growth hormone affected. With regard to RA pro-apoptotic activity, they describe an increase in caspase-3 activity in AtT-20 cells, decreasing their viability and

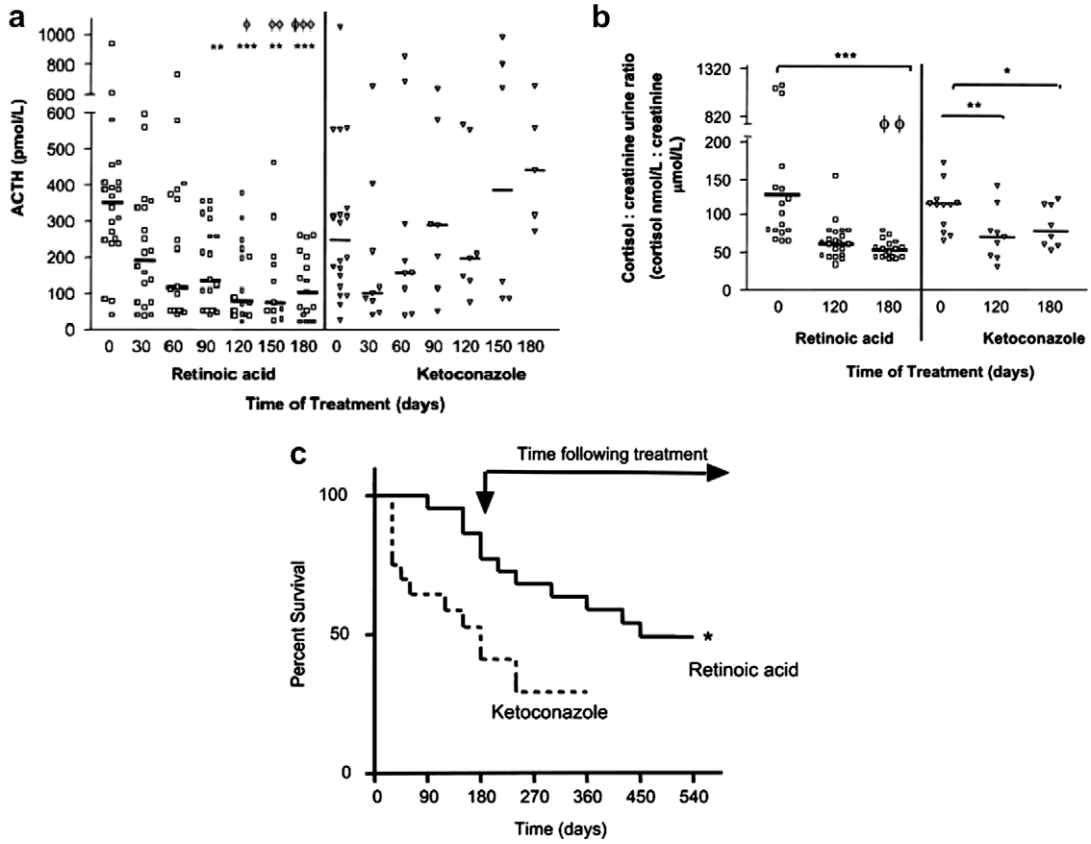

Fig. 2. Changes in ACTH (a); cortisol:creatinine urinary ratio (b) and survival curves (c) in dogs with Cushing's disease treated with 2 mg/kg body weight/day of retinoic acid (isotretinoin 9-*cis*) *vs.* 20 mg/kg weight/day of ketoconazole. At 120 days, ACTH decrease (a) and that of the urinary cortisol: creatinine ratio (b) is evident, indicating that the drug inhibited ACTH synthesis and consequently cortisol production (evaluated through its urinary excretion). Survival (c) is greater in dogs treated with isotretinoin 9-cis, both during the treatment and after treatment removal. *Open squares* (Rx) and *triangles* (Ktz) represent individual dogs and their variation during treatment. (Taken from: Castillo et al., 2006. Permission from Endocrinology, copyright 2006, The Endocrine Society).

proliferation. It has been observed in *in vivo* studies in nu/nu mice that if AtT-20 cells were treated with RA before implantation, the corticotrophinoma did not develop and no changes were seen in ACTH and cortisol concentrations of the mice. The opposite happened in those that were inoculated with untreated cells. Studying the effect of RA on previously formed tumours, it was observed that tumour growth was inhibited in mice under RA treatment, showing focal signs of cell death on histopathological examination. Both ACTH and corticosterone had lower concentrations than that of controls. The authors conclusion is that RA inhibits ACTH synthesis by inhibiting POMC transcription because of its activity on AP-1 and Nur77/Nurr1, and reduces the proliferation and survival of the corticotrophinoma; thus having potential therapeutic use in CD. The report by Páez-Pareda et al. (2001) was verified in a recent study carried out in 22 dogs with PDH. Dogs treated with RA-9 cis (2 mg/kg/day) showed clinical improvement (decrease of liquids and solid food intake; oestrous cycle recovery, longer survival). Likewise, magnetic resonance imaging (MRI) revealed a reduction in tumour size; and the decrease in ACTH and plasma α-MSH and in the urinary cortisol/creatinine ratio was also evident (Fig. 2) (Castillo et al., 2006).

5.3. Cabergoline (Cbg)

Dopamine exerts an inhibitory effect on the adenohypophysis and pars intermedia through its union with D2 type receptors (D2R). Dopaminergic agonists can be used in corticotrophinoma treatment as 80% express these receptors, and cabergoline, being the most efficient of these agonists is proposed to be useful in 40% of the corticotrophinomas (Colao et al., 2000; Pivonello et al., 2004). We have recently communicated that 42% (17/40) of dogs treated with cabergoline (0.07 mg/kg) every 48 h not only improve clinical signs (decrease of liquids and solid food intake; oestrous cycle recovery, reduction in abdomen size, longer survival) but also show a decline in tumour size as well as a decrease in plasma ACTH and α-MSH concentrations and in the cortisol/creatinine ratio in urine (Fig. 3) (Castillo et al., 2008).

6. Discussion

Diverse and multiple associated factors take part in the origin of the corticotrophinoma, with the monoclonal theory being the most widely accepted (Assa and Ezzat, 1998; Clayton and Farrell, 2001; Herman et al., 1990; Levy and Lightman, 2003; Melmed, 2003). An hypothalamic desregulation initiating the tumour process from a first phase of hyperplasia should not be totally discarded (Dahia and Grossman, 1999; Hereñú et al., 2006a,b; Otte et al., 2005; Teshima et al., 2009b). Although Van Wijk et al. (1992) did not observe correlation between CRH concentration in cerebrospinal fluid and ACTH plasma concentration in dogs with PDH, the study was carried out in animals of up to 11 years of age with corticotrophinoma and does not take into account older dogs, where dopaminergic neurodegeneration would be an important factor. Also, the fact that these dogs already had an ACTH secreting tumour makes it impossible to totally discard the previous action of CRH, at least in some of the cases, as CRH could have been inhibited by the ACTH and cortisol increase. On the other hand, LOH, mutation or defects in factors controlling the cell cycle would lead to tumour growth commencing with the affected cell (clone) (Dahia and Grossman, 1999; Levy and Lightman, 2003). Firm candidates for this process are the Rb1 gene, proteins p27, p21 and p16 (Dahia and Grossman,

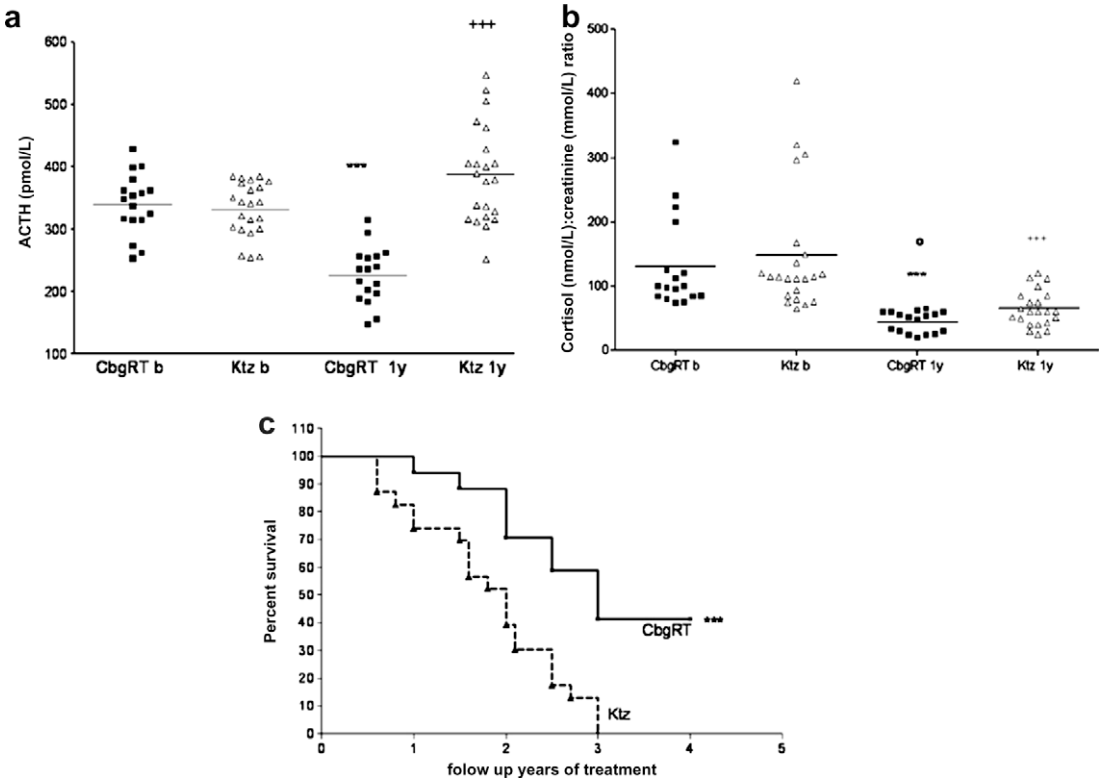

Fig. 3. Changes in ACTH (a), cortisol: creatinine urinary ratio (b) and survival curves (c) in dogs with Cushing's disease treated every 48 h with 0.07 μg/kg of cabergoline. Evaluation a year later of the dogs that responded to cabergoline (CbgRT) showed lower ACTH concentrations and a lower cortisol/creatinine ratio in urine when compared to the controls (Ktz). Survival was greater in CbgRT, reaching up to 5 years in dogs treated with the drug. (Taken from: Castillo et al., 2008. Research in Veterinary Science, copyright 2008).

1999; Levy and Lightman, 2003; Lidhar et al., 1999) and defects in GR, Nur77/Nurr1 and 11 beta hydroxysteroid dehydrogenase (Kang, 2000; Kovalovsky et al., 2002; Lamberts, 2002; Levy and Lightman, 2003; Martens et al., 2005; Teshima et al., 2009b). More studies are necessary to know if BMPs have a role in tumour pathogenesis, given their interaction with "home-box" Ptx-1 and Tpit genes and with RXR/RAR activity (Merino and Hurlé, 2003; Nudi et al., 2005; Vallette-Kasic et al., 2003; Giacomini et al., 2006). Light shed from studies by Kovalovsky et al. (2002) and Páez-Pareda et al. (2001) on Nur77/Nurr1 make it evident that the greater expression of its promoter gene would lead to an amplification of CRH activity with an increased synthesis of POMC and trophic effects on this peptide. It remains to be seen if this greater expression, similar to that of the PPAR-γ, is due to mutations or LOH inducing tumour genesis. These therapeutic alternatives open up an interesting field of new options. Due to the scarce adverse effects they have demonstrated, these therapies would be of great use. They could be indicated as the initial treatment in cases where surgery is contraindicated or as a therapeutic aid to surgery (post surgical indication) or pituitary radiotherapy.

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References

Agarwal, M.K., 1996. The antiglucocorticoid action of mifepristone. Pharmacology and Therapeutics 70, 183–213.

Ambrosi, B., Aroiso, M., Dall'Asta, C., Cannavo, S., Libe, R., Vigo, T., Epaminonda, P., Chiodini, I., Ferrero, S., Trimarchi, F., Arosio, M., Beck-Peccoz, P., 2004. Effects of the chronic administration of the PPAR-γ receptor ligand, rosiglitazone, in Cushing's disease. European Journal of Endocrinology 151, 173–178.

Akita, S., Webster, J., Ren, S.G., Takino, H., Said, J., Zand, O., Melmed, S., 1995. Human and murine pituitary expression of leukemia inhibitory factor. Novel intrapituitary regulation of adrenocorticotropin hormone synthesis and secretion. Journal of Clinical Investigation 95, 1288–1298.

Arnaldi, G., Cardinaletti, M., Boscaro, M., 2007. Advances in medical treatment of Cushing's disease. Expert Review Endocrinology and Metabolism 2, 735–743.

Assa, S.L., Ezzat, S., 1998. The cytogenesis and pathogenesis of pituitary adenomas. Endocrine Reviews 19, 798–827.

Barker, E.N., Campbell, S., Tebb, A.J., Neiger, R., Herrtage, M.E., Reid, S.W., Ramsey, I.K., 2005. A comparison of the survival times of dogs treated with mitotane or trilostane for pituitary-dependent hyperadrenocorticism. Journal of Veterinary Internal Medicine 19, 810–815.

Boscaro, M., Barzon, L., Fallo, F., Sonino, N., 2001. Cushing's syndrome. The Lancet 357, 783–790.

Brada, M., 1993. The long term efficacy of conservative surgery and radiotherapy in the control of pituitary adenomas. Journal of Clinical Endocrinology 38, 571–578.

Braddock, J.A., Church, D.B., Robertson, I.D., Watson, A.D., 2004. Inefficacy of selegiline in treatment of canine pituitary-dependent hyperadrenocorticism. Australian Veterinary Journal 82, 272–277.

Bruyette, D.S., 1995. Canine pituitary-dependent hyperadrenocorticism: a spontaneous animal model for neurodegenerative disorders and their treatment with L-deprenyl. In: Yu, P., Tipton, K., Boulton, A., (Eds.), Current Neurochemical and Pharmacological Aspects of Biogenicamines, Progress in Brain Research, vol. 106, pp. 207–215.

Buckley, N., Bates, A.S., Broome, R.C., Strange, R.C., Perret, C.W., Burke, C.W., Clayton, R.N., 1995. P53 protein accumulates in Cushings adenomas and invasive non-functional adenomas. Journal of Clinical Endocrinology and Metabolism 79, 1513–1516.

Castillo, V.A., Lalia, J.C., Casal, J., Casal, G., Esarte, M., Mira, G., Rodriguez, M., Marquez, A., 1996. Aminoglutetimide: alternativa terapéutica en caninos con Enfermedad de Cushing (hipófiso-dependiente). Avances en Medicina Veterinaria 11, 93–96.

Castillo, V.A., Giacomini, D.P., Paez-Pereda, M., Stalla, J., Labeur, M., Theodoropoulou, M., Holsboer, F., Grossman, A., Stalla, G., Arzt, E., 2006. Retinoic acid as a novel medical therapy for Cushing's disease in dogs. Endocrinology 174, 4438–4444.

Castillo, V.A., Gomez, N.V., Lalia, J.C., Cabrera Blatter, M.F., Garcia, J.D., 2008. Cushinǵs disease in dogs: Cabergoline treatment. Research in Veterinary Science 85, 26–34.

Chanson, P., Salenave, S., 2004. Diagnosis and treatment of pituitary adenomas. Minerva Endocrinology 29, 241–273.

Chen, Y., Hu, X., Wei, L., 2004. Molecular interaction of retinoic acid receptor with coregulators PCAF and RIP140. Molecular and Cellular Endocrinology 226, 43–50.

Clayton, R.N., Farrell, W.E., 2001. Clonality of pituitary tumours: more complicated than initially envisaged? Brain Pathology 11, 313–327.

Colao, A., Lombardi, G., Annunziato, L., 2000. Cabergoline. Experimental Opinion Pharmacotherapy 1, 555–574.

Dahia, P.L., Grossman, A.B., 1999. The molecular pathogenesis of corticotroph tumors. Endocrine Reviews 20, 136–155.

Dasen, J.S., O' Conell, S.M., Flynn, S.E., Treier, M., Gleiberman, A.S., Szeto, D.P., Hooshmand, F., Aggarwal, A., Rosenfeld, M.G., 1999. Reciprocal interactions of Pit1 and GATA2 mediate signaling gradient-induced determination of pituitary cell type. Cell 97, 587–596.

de Bruin, C., Hanson, J.M., Meij, B.P., Kooistra, H.S., Waaijers, A.M, Uitterlinden, P, Lamberts,S.W.J., Hofland, L.J., 2008. Expression and functional analysis of dopamine receptor subtype 2 and somatostatin receptor subtypes in canine. Cushing's Disease Endocrinology 149, 4357–4366.

den Hertog, E., Braakman, J.C., Teske, E., Kooistra, H.S., Rijnberk, A., 1999. Results of non-selective adrenocorticolysis by o, p'-DDD in 129 dogs with pituitary-dependent hyperadrenocorticism. The Veterinary Record 144, 12–17.

Dieterich, K.D., Guldenkifinger, E.D., Lüdecke, D.K., Lehnert, H., 1998. Mutation and expression analysis of corticotropin-releasing factor 1 receptor in adrenocorticotropin-secreting pituitary adenomas. Journal of Clinical Endocrinology and Metabolism 83, 3327–3331.

Engelhardt, D., Weber, M.M., 1994. Therapy of Cushinǵs Syndrome with steroid biosyntesis inhibitors. The Journal of Steroid Biochemistry and Molecular Biology 49, 261–267.

Engler, D., Redei, E., Kola, I., 1999. The corticotropin-release inhibitory factor hypothesis: a review of the evidence for existence of inhibitory as well as stimulatory hypophysiotropic regulation of adrenocorticotropin secretion and biosynthesis. Endocrine Reviews 20, 460–500.

Evans, C.O., Young, A.N., Brown, M.R., Brat, D.J., Parks, J.S., Neish, A.S., Oyesiku, N.M., 2001. Novel pattern of gene expression in pituitary adenomas identified by complementary deoxyribonucleic acid microarrays and quantitative reverse transcription-polymerase chain reaction. Journal of Clinical Endocrinology and Metabolism 86, 3097–3107.

Fracassi, F., Gandini, G., Diana, A., Preziosi, R., van den Ingh, T.S., Famigli-Bergamini, P., Kooistra, H.S., 2007. Acromegaly due to somatroph adenoma in a dog. Domestic Animal Endocrinology 32, 43–54.

Giacomini, D., Paez-Pereda, M., Theodoropoulou, M., Labeur, M., Refojo, D., Gerez, J., Chervin, A., Berner, S., Losa, M., Buchfelder, M., Renner, U., Stalla, G.K., Arzt, E., 2006. Bone morphogenetic protein-4 inhibits corticotroph tumor cells: involvement in the retinoic acid inhibitory action. Endocrinology 147, 247–256.

Gsponer, J., De Tribolet, N., Déruaz, J.P., Janzer, R., Uské, A., Mirimanoff, R.O., Reymond, M.J., Rey, F., Temler, E., Gaillard, R.C., Gomez, F., 1999. Diagnosis, treatment and outcome of pituitary tumors and other abnormal intracellular masses. Retrospective analysis of 353 patients. Medicine (Baltimore) 78 (23), 6–269.

Hanson, J.M., Van't, H.M., Voorhout, G., Teske, E., Kooistra, H.S., Meij, B.P., 2005. Efficacy of transsphenoidal hypophysectomy in treatment of dogs with pituitary-dependent hyperadrenocorticism. Journal of Veterinary Internal Medicine 19, 687–694.

Hanson, J.M., Teske, E., Voorhout, G., Galac, S., Kooistra, H.S., Meij, B.P., 2007. Prognostic factors for outcome after transsphenoidal hypophysectomy in dogs with pituitary-dependent hyperadrenocorticism. Journal of Neurosurgery 107, 830–840.

Hanson, J.M., Mola, J.A., Leegwater, P.A.J., Bilodeau, S., Drouin, J., Meij, B.P., 2008. Expression and mutation analysis of Tpit in the canine pituitary gland and corticotroph adenomas. Domestic Animal Endocrinology 34, 217–222.

Heaney, A.P., Fernando, M., Melmed, S., 2002a. Functional role of estrogen in pituitary tumor pathogenesis. Journal of Clinical Investigation 109, 277–283.

Heaney, A.P., Fernando, M., Yong, W., Melmed, S., 2002b. Functional PPAR-γ receptor is a novel therapeutic target for ACTH-secreting pituitary adenomas. Nature Medicine 8, 1281–1287.

Heaney, A.P., Fernando, M., Melmed, S., 2003. PPAR-γ receptor ligands: novel therapy for pituitary adenomas. Journal of Clinical Investigation 111, 1381–1388.

Hereñú, C., Brown, O., Sosa, Y., Morel, G., Reggiani, P., Bellini, M., Goya, R., 2006a. The neuroendocrine system as a model to evaluate experimental gene therapy. Current Gene Therapy 6, 125–129.

Hereñú, C., Rimoldi, O., Becú-Villalobos, D., Cambiaggi, D., Portiansky, E., Goya, R., 2006b. Restorative effect of insulin-like growth factor-I gene therapy in the hypothalamus of senile rats with dopaminergic dysfunction. Gene Therapy 13, 1–9.

Herman, V., Fagin, J., Gonsky, R., Kovacs, K., Melmed, S., 1990. Clonal origin of pituitary adenomas. Journal of Clinical Endocrinology and Metabolism 71, 1427–1433.

Jacks, T., Fazeli, A., Schmitt, E.M., 1999. Effects of an Rb mutation in the mouse. Nature 359, 295–300.

Kang, H., 2000. Retinoic acid and its receptors repress the expression and transactivation functions of Nurr77: a possible mechanism for the inhibition of apoptosis by retinoic acid. Experimental Cell Research 256, 545–554.

Karl, M., Lamberts, S.W., Koper, J.W., Katz, D.A., Huizenga, N.E., Kino, T., Haddad, B.R., Hughes, M.R., Chrousos, G.P., 1996a. Cushing's disease preceded by generalized glucocorticoid resistance. clinical consequences of a novel dominant negative

glucocorticoid receptor mutation. Proceedings of the Association of American Physicians 108, 296–307.
Karl, M., Von Wichert, G., Kempter, E., Katz, D.A., Reincke, M., Monig, H., Ali, I.U., Stratakis, C.A., Oldfield, E.H., Chrousos, G.P., Schulte, H.M., 1996b. Nelson's Syndorme associated with somatic frame shift mutation in the glucocorticoid receptor gene. Journal of Clinical Endocrinology and Metabolism 81, 124–129.
Kintzer, P., Peterson, M., 1991. Mitotane (o,ṕ-DDD) treatment of 200 dogs with PDH. Journal Veterinary Internal Medicine 5, 182–190.
Kovalovsky, D., Refojo, D., Liberman, A.C., Hochbaum, D., Paez Pereda, M., Coso, O.A., Stalla, G.K., Holsboer, F., Arzt, E., 2002. Activation and induction of Nur77/Nurr1 in corticotrophs by CRH/cAMP: involvement of calcium, protein kinase A and MAPK pathways. Molecular Endocrinology 16, 1638–1651.
Lamberts, S.W., 2002. Glucocorticoid receptors and Cushing's disease. Molecular and Cellular Endocrinology 197, 69–72.
Lamolet, B., Poulin G., Chu, K., Guillemot, F., Tsai, M.J., Drouin, J., 2004.Tpit-independent function of NeuroD1(BETA2) in pituitary corticotroph differentiation. Molecular Endocrinology 18, 995–1003.
Lanctôt, C.h., Gauthier, Y., Drouin, J., 1999. Pituitary homebox 1 (Ptx1) is differentially expressed during pituitary development. Endocrinology 140, 1416–1422.
Lei, M., Thé, H., 2003. Retinoids and retinoic acid receptor in cancer. European Journal of Cancer Supplements 1, 13–18.
Levy, A., Lightman, S., 2003. Molecular defects in the pathogenesis of pituitary tumors. Frontiers in Neuroendocrinology 24, 94–127.
Lidhar, K., Korbonits, M., Jordan, S., Khalimova, Z., Kaltsas, G., Lu, X., Clayton, R.N., Jenkins, P.J., Monson, J.P., Besser, G.M., Lowe, D.G., Grossman, A.B., 1999. Low expression of the cell cycle inhibitor p27/kip1 in normal corticotroph cells, corticotroph tumors and malignant pituitary tumors. Journal of Clinical Endocrinology and Metabolism 84, 3823–3830.
Lovekamp-Swan, T., Chaffin, C.L., 2005. The peroxisome proliferator-activated receptor γ ligand troglitazone induces apoptosis and p53 in rat granulosa cells. Molecular and Cellular Endocrinology 233, 15–24.
Maira, M., Martens, C., Philips, A., Drouin, J., 1999. Heterodimerization between members of the Nurr subfamily of orphan nuclear receptor as a novel mechanism for gen activation. Molecular and Cellular Biology 19, 7549–7557.
Mampalam, T.J., Tyrrell, J.B., Wilson, C.B., 1988. Transphenoidal microsurgery for Cushing's disease: a report of 216 cases. Annals of Internal Medicine 109, 487–493.
Martens, C.H., Bilodeau, S., Maira, M., Gauthier, Y., Drouin, J., 2005. Protein-protein interactions and transcriptional antagonism between the subfamily of NGFI-B/Nurr77 orphan nuclear receptors and glucocorticoid receptor. Molecular Endocrinology 19, 885–897.
Meij, B.P., Mol, J.A., Bevers, M.M., Rijnberk, A., 1997. Residual pituitary function after transsphenoidal hypophysectomy in dogs with pituitary-dependent hyperadrenocorticism. Journal Endocrinology 155, 531–539.
Meij, B.P., 2001. Hypophysectomy as a treatment for canine and feline Cushinǵs disease. Veterinary Clinics of North America 31, 1015–1041.
Meij, B., Voorhout, G., Rijnberk, A., 2002. Progress in transsphenoidal hypophysectomy for treatment of pituitary-dependent hyperadrenocorticism in dogs and cats. General and Comparative Endocrinology 197, 89–96.
Melmed, S., 2003. Mechanisms for pituitary tumorigenesis: the plastic pituitary. Journal of Clinical Investigation 112, 1603–1618.
Merino, R., Hurlé, J.M., 2003. The molecular basis of retinoid action in tumors. Trends in Molecular Medicine 12, 508–511.
Nakayama, K., Ishida, N., Shirane, M., Inomata, A., Inoue, T., Shishido, N., Horii, I., Loh, D.Y., Nakayama, K., 1996. Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia and pituitary tumors. Cell 85, 707–720.
Nudi, M., Ouimette, J.F., Drouin, J., 2005. Bone morphogenic protein (Smad)-mediated repression of proopiomelanocortin transcription by interference with Pitx/Tpit activity. Molecular Endocrinology 19, 1329–1342.
Ooi, G.T., Tawadros, N., Escalona, R.M., 2004. Pituitary cell lines and their endocrine applications. Molecular and Cellular Endocrinology 228, 1–21.
Orth, D.N., Kovacs, W., 1998. The adrenal cortex. In: Williams Textbooks of Endocrinology, ninth ed. W.B. Saunders Company, Philadelphia, pp. 569–572.
Otte, C., Lenoci, M., Metzler, T., Yehuda, R., Marmar, M.R., Neylan, T.C., 2005. Hypothalamic–pituitary–adrenal axis activity and sleep in posttraumtic stress disorder. Neuropsychopharmacology 6, 1173–1180.
Páez-Pereda, M., Missale, C., Grübler, Y., Arzt, E., Scaf, L., Stalla, G.K., 2000. Nerve growth factor and retinoic acid inhibit proliferation and invasion in thyroid tumor cells. Molecular and Cellular Endocrinology 167, 99–106.
Páez-Pareda, M., Kovalovsky, D., Hopfner, U., Theodoropoulou, M., Pagotto, U., Uhl, E., Losa, M., Stalla, J., Grübler, Y., Missale, C., Arzt, E., Stalla, G.K., 2001. Retinoic acid prevents experimental Cushing syndrome. Journal of Clinical Investigation 108, 1123–1131.
Petrossians, P., Ronci, N., Valdes Socin, H., Kalife, A., Stevenaert, B., Bloch, B., Tabarin, A., Beckers, A., 2000. ACTH silent adenoma shrinking under cabergoline. European Journal of Endocrinology 144, 51–57.
Philips, A., Lesage, S., Gingras, R., Maira, M.H., Gauthier, Y., Hugo, P., Douin, J., 1997. Novel dimeric Nur77 signaling mechanism in endocrine and lymphoid cells. Molecular and Cellular Biology 17, 5946–5951.
Pivonello, R., Ferone, D., de Herder, W., Kros, J., de Caro, M., Arvigo, M., Annunziato, L., Lombardi, G., Colao, A., Ho, L., Lamberts, S., 2004. Dopamine receptor expression and function in corticotroph pituitary tumors. Journal of Clinical Endocrinology and Metabolism 89, 2452–2462.
Reichlin, S., 1998. Neuroendocrinology. In: Williams Textbooks of Endocrinology, ninth ed. W.B. Saunders Company, Philadelphia, pp. 165–208.
Reine, N.J., 2007. Medical management of pituitary-dependent hyperadrenocorticism: mitotane VS trilostane. Clinical Techniques in Small Animal Practice 22, 18–25.
Reusch, C.E., Steffen, T., Hoerauf, A., 1999. The efficacy of l-deprenyl in dogs with pituitary-dependent hyperadrenocorticism. Journal Veterinary Internal Medicine 13, 291–301.
Ruckstuhl, N.S., Nett, C.S., Reusch, C.E., 2002. Results of clinical examinations, laboratory tests, and ultrasonography in dogs with pituitary-dependent hyperadrenocorticism treated with trilostane. American Journal of Veterinary Research 63, 506–512.
Savage, J.J., Yaden, B.C., Kiratipranon, P., Rhodes, S.J., 2003. Transcriptional control during mammalian anterior pituitary development. Gene 319, 1–19.
Seaholtz, A., 2000. Regulation of adrenocorticotropic hormone secretion: lessons from mice deficinet in corticotropin-realising hormone. Journal of Clinical Investigation 105, 1187–1188.
Sieber-Ruckstuhl, N., Boretti, F., Wenger, M., Maser-Gluth, C., Reusch, C., 2006. Cortisol, aldosterone, cortisol precursor, androgen and endogenous ACTH concentrations in dogs with pituitary-dependant hyperadrenocorticism treated with trilostane. Domestic Animal Endocrinology 31, 63–75.
Sieber-Ruckstuhl, N.S., Boretti, F.S., Wenger, M., Maser-Gluth, C., Reusch, C.E., 2008. Serum concentration of cortisol and cortisone in healthy dogs and dogs with pituitary-dependent hyperadrenocorticism treated with trilostane. The Veterinary Record 163, 477–481.
Simmons, N.E., Alden, T.D., Thorner, M.O., Laws Jr., E.R., 2001. Serum cortisol response to transphenoidal surgery for Cushing's disease. Journal of Neurosurgery 95, 1–8.
Sonino, N., Fava, G.A., Boscaro, M., 1993. A role for life events in the pathogenesis of Cushing's disease. Clinical Endocrinology (Oxford) 38, 261–264.
Sonino, N., Fava, G.A., Fallo, F., Franceschetto, A., Belluardo, P., Boscaro, M., 2000. Effect of the serotonin antagonists ritanserin and ketanserin in Cushing's disease. Pituitary 3, 55–59.
Sugawara, A., Yen, P.M., Qi, Y., Lechan, R.M., Chin, W.W., 1995. Isoform-specific retinoid-X receptor (RXR) antibodies detect differential expression of RXR proteins in the pituitary gland. Endocrinology 136, 1766–1774.
Suh, H., Gage, P.J., Drouin, J., Camper, S.A., 2002. Pitx2 is required at multiple stages of pituitary organogenesis: pituitary primordium formation and cell specification. Development 129, 329–337.
Suri, D., Weiss, R., 2005. Effect of pioglitazone on adrenocorticotropic hormone and cortisol secretion in Cushing's disease. Journal of Clinical Endocrinology and Metabolism 90, 1340–1349.
Tanaka, S. 2003.Comparative aspects of intracellular proteolytic processing of peptide hormone precursors: studies of proopiomelanocortine processing. Zoological Science 20, 1183–1198.
Teshima, T., Hara, Y., Takekoshi, S., Nezu, Y., Harada, Y., Yogo, T., Teramoto, A., Osamura, R.B., Tagawa, M., 2009a. Trilostane-induced inhibition of cortisol secretion results in reduced negative feedback at the hypothalamic–pituitary axis. Domestic Animal Endocrinology 36, 32–44.
Teshima, T., Hara, Y., Takekoshi, S., Teramoto, A., Osamura, R.Y., Tagawa, M., 2009b. Expression of genes related to corticotropin production and glucocorticoid feedback in corticotroph adenomas of dogs with Cushing's disease. Domestic Animal Endocrinology 36, 3–12.
Thibonnier, M., Preston, J.A., Dulin, N., Wilkins, P.L., Berti-Mattera, L.N., Mattera, R., 1997. The human V3 pituitary vasopressin receptor: ligand binding profile and density-dependent signaling pathways. Endocrinology 138, 4109–4122.
Treier, M., Gleiberman, A.S., O' Conell, S.M., Szeto, D.P., McMahon, J.A., McMahon, A.P., Rosenfeld, M.G., 1998. Multistep signaling requirements for pituitary organogenesis in vivo. Genes and Development 12, 1691–1704.
Vallette-Kasic, S., Figarella-Branger, D., Grino, M., Pulichino, A.M., Dufour, H., Grisoli, F., Enjalbert, A., Drouin, J., Brue, T., 2003. Differential regulation of proopiomelanocortin and pituitary-restricted transcription factor (TPIT), a new marker of normal and adenomatous human corticotrophs. Journal of Clinical Endocrinology and Metabolism 88, 2050–3056.
Van Wijk, P.A., Rijnberk, A.D., Croughs, R.J.M., Voorhout, G., Sprang, E.P.M., Mol, J.A., 1992. Corticotropin-releasing hormone and adrenocorticotropic hormone concentrations in cerebrospinal fluid of dogs with pituitary dependent hyperadrenocorticism. Endocrinology 131, 2659–2662.
Wenger, M., Sieber-Ruckstuhl, N.S., Müller, C., Reusch, C.E., 2004. Effect of trilostane on serum concentrations of aldosterone, cortisol, and potassium in dogs with pituitary-dependent hyperadrenocorticism. American Journal of Veterinary Research 65, 1245–1250.
Yano, H., Readhead, C., Nakashima, M., Ren, S., Melmed, S., 1998. Pituitary-directed leukaemia inhibitory factor transgene causes Cushing/s Syndrome neuro-immune-endocrine modulation of pituitary development. Molecular Endocrinology 12, 1708–1720.
Zeugswetter, F., Hoyer, M.T., Pagitz, M., Benesch, T., Hittmair, K.M., Thalhammer, J.G., 2008. The desmopressin stimulation test in dogs with Cushing's syndrome. Domestic Animal Endocrinology 34, 254–260.
Zhang, X., Horwitz, G.A., Heaney, A.P., Nakashima, M., Prezant, T.R., Bronstein, M.D., Melmed, S., 1999. Pituitary tumor transforming gene (PTTG) expression in pituitary adenomas. Journal of Clinical Endocrinology and Metabolism 84, 761–767.
Zhou, X.F., Shen, X.Q., Shemshedim, L., 1999. Ligand activated retinoic acid receptor inhibits AP-1 transactivation by disrupting c-Jum/c-Fos dimerization. Molecular Endocrinology 13, 276–285.