J Steroid Biochem Molec Biol Vol 43, No 5, pp 379–384, 1992
Printed in Great Britain

0960-0760/92 $5 00 + 0 00

UNRESTRAINED PRODUCTION OF PROOPIOMELANOCORTIN (POMC) AND ITS PEPTIDE FRAGMENTS BY PITUITARY CORTICOTROPH ADENOMAS IN CUSHING'S DISEASE

XAVIER BERTAGNA
Centre de Recherche sur les Maladies Endocriniennes, CHU Cochin, 24, rue du Faubourg Saint Jacques, 75014 Paris, France

Summary—The hallmark of ACTH oversecretion in Cushing's disease is its partial resistance to the normal suppressive effect of glucocorticoids Because ACTH secretion by the pituitary tumor is not normally restrained ACTH is overproduced with subsequent chronic hypercortisolism Since peripheral tissues have retained their normal sensitivity to the action of cortisol they appropriately develop the features of Cushing's disease The question of whether a collection of corticotroph cells, eventually arranged in an adenomatous-like fashion, is a primary pituitary event or is corticotropin-releasing factor driven has had no response so far Clonal composition of such lesions has been determined by X chromosome inactivation using DNA probes which detect multiallelic polymorphism in females A monoclonal pattern is found in all macroadenomas ACTH is co-secreted with other peptide fragments derived from their common polypeptide precursor, proopiomelanocortin (POMC) As a rule POMC processing in pituitary tumors is qualitatively unaltered plasma values of the N-terminal fragment, the joining peptide, the β- and γ-lipotropins, and β-endorphin all are valid alternate markers of the tumor activity Tumor POMC peptides including ACTH and its phosphorylated form usually show no peculiar or unexpected molecular forms in contrast with what is often found when POMC expression occurs in a non-pituitary tumor

In Cushing's disease chronic cortisol excess is due to pituitary ACTH oversecretion In the vast majority of patients a corticotroph adenoma is present the activity of which is not normally restrained by the negative feed-back effect of cortisol

1 PROOPIOMELANOCORTIN (POMC) GENE EXPRESSION IS NOT QUALITATIVELY ALTERED IN CORTICOTROPH ADENOMAS

In the vast majority of corticotroph adenomas the products of POMC gene transcription and of POMC processing are similar to those in the normal human anterior pituitary

The gene transcription, shows no gross abnormality and the POMC transcripts in pituitary tumors are similar to those in the normal pituitary [1–4] a 1200 nt POMCmRNA is the highly predominant—if not sole—transcript A small percentage (<5%) of transcripts result from an alternate mode of RNA splicing adding 30 nt at the 5′ end of the second exon It has no implication on the open reading frame which is not modified Fewer than 1% of transcripts result from the use of an upstream promoter at −369 nt [5]

The N-terminal fragment [6], the joining peptide [7], authentic $ACTH_{1-39}$ [8–11], β-lipotropin (LPH) and variable amounts of γ-LPH and β-endorphin [11–13] are the normal end-products of POMC processing found both in tumor extracts and in culture media A somewhat higher proportion of β-endorphin over β-LPH—and γ- over β-LPH—has been reported [1, 14] Yet the recruitment of proteolytic sites which are not normally activated in the normal pituitary is not observed and peptides like corticotropin-like intermediary lobe peptide and α-melanocyte stimulating hormone are neither formed nor released This general finding supports the use of highly specific immunoradiometric assays for plasma ACTH detection as a valid and significant means to evaluate patients with Cushing's disease [15] In rare instances qualitative alterations of POMC gene expression have been described, in silent corticotroph adenomas and in pituitary cancers [16–18]

Proceedings of the XV Meeting of the International Study Group for Steroid Hormones, Rome, Italy, 28–30 November 1991

Thus tumor POMC peptides, including ACTH, usually show no peculiar or unexpected molecular forms in contrast with what is often found when POMC expression occurs in a non-pituitary tumor Any of them can be used alternatively for the clinical investigation, all their plasma values being highly correlated [19]

2 POMC GENE EXPRESSION IS NOT NORMALLY RESTRAINED IN CORTICOTROPH ADENOMAS

In a system normally regulated by a negative feed-back loop two determinants which are not exclusive of each other may theoretically provoke and maintain unrestrained hormone production the set-point defect at the cell level, and the tumoral mass at the tissue level These pathophysiological mechanisms have been thoroughly studied *in vivo* and *in vitro* in various models such as primary hyperparathyroidism where the two determinants cooperate [20] In the case of human corticotroph tumors *in vitro* studies offer obvious difficulties the latter tumors are much rarer, and direct comparison between the tumoral and the normal corticotroph cell is seldom achieved Yet a number of experimental and human studies offer the grounds on which can stem a tentative—and speculative—pathophysiological explanation of the phenomenon

ACTH oversecretion in Cushing's disease is characterized by its partial resistance to the normal suppressive effect of glucocorticoids [21, 22] The dose–response curve between administered dexamethasone and plasma ACTH or endogenous cortisol production is shifted to the right Because ACTH secretion by the pituitary tumor is not normally restrained ACTH is overproduced with subsequent chronic hypercortisolism Since peripheral tissues have retained their normal sensitivity to the action of cortisol [23, 24] they appropriately develop the features of Cushing's disease

In vitro studies have confirmed that pituitary corticotroph adenomas are not autonomous and have indeed retained some sensitivity to the suppressive effect of glucocorticoids which invariably decrease basal and/or stimulated ACTH release [25–31] A direct comparison between the responses of normal and tumoral cells *in vitro* is lacking most of the time A single study measured the effects of two doses of dexamethasone (1 and 10 μg/dl) on both ACTH release and POMCmRNA content in cultured cells obtained either from corticotroph adenomas or from their, presumably normal, peri-adenomatous tissues whereas dexamethasone efficiently reduced both parameters in the peri-adenomatous cells, its suppressive effect was reduced in the tumoral cells [2]

Schematically normal secretion of ACTH results from a fine equilibrium within the corticotroph cell between two opposite regulators with stimulatory [cAMP and protein kinase C pathways driven by corticotropin-releasing factor (CRF) and arginine vasopressin (AVP)] and inhibitory (glucocorticoid pathway) actions, a subtle imbalance between the two regulators should lead to ACTH dysregulation and, in the case of overproduction to an apparent state of resistance to glucocorticoids Thus variable, and probably numerous, mechanisms may provoke a set-point defect

A gross abnormality in the nature of the glucocorticoid receptor in the tumoral corticotroph cell has not yet been demonstrated [32] The recent elucidation of a molecular alteration responsible for the syndrome of general resistance to glucocorticoids [33] may pinpoint more precise targets for future studies on the DNA and/or mRNA coding for the human glucocorticoid receptor in the tumor Alternatively the functional activity of a structurally normal glucocorticoid receptor may be reduced by a variety of intracellular defects among many other causes, it is decreased in experimental animal models where v-*mos* and Ha-*ras* oncoproteins are overexpressed [34, 35], and recent data have elucidated a general mechanism whereby the activated glucocorticoid receptor and the products of the proto oncogenes c-*fos* and c-*jun* inhibit each other's action at the gene level [36, 37]

A set-point defect might be caused as well by the exaggerated activation of cAMP and/or protein kinase C pathways *In vitro* studies on rat anterior pituitary cells show that whenever one of these two pathways is stimulated, ACTH suppression by glucocorticoids is diminished [38–40] Increased cAMP formation in AtT-20 cells directly blunts the suppressive effect of glucocorticoids on POMC synthesis through the inhibition of glucocorticoid receptor binding to DNA [41] A subset of human GH-producing pituitary tumors is associated with increased production of cAMP [42] it has been shown to result from the intrinsic activation of their Gs protein by a single base mutation which suppresses the GTPase activity

of the α subunit [43] This precise type of acquired genetic alteration has not, so far, been found in corticotroph tumors [44] An alternate hypothesis, already suggested for other types of endocrine tumors, is that the tumoral cell acquires an abnormal sensitivity to non-CRF hypothalamic neuro-hormones [45] *In vivo* studies have claimed that various hypothalamic factors like thyrotropin-releasing hormone (TRH) and luteinizing hormone-releasing hormone (LHRH) would increase ACTH release in occasional patients [46–48] The significance of these results suffer from the inescapable drawback of uncontrolled trials Very few studies reported the data *in vivo* and *in vitro* in the same patient Few cases however have been described which still make it possible that some rare tumor has acquired this unexpected sensitivity [27, 49, 50] More and more growth factors also seem to play a role in an autocrine or paracrine fashion within the pituitary [51], their involvement in the pathogenesis of tumor formation is not yet established

The mass of the tumor is another determinant of the final level of ACTH oversecretion *In vitro* studies on rat anterior pituitary cells show that increasing glucocorticoids cannot totally suppress the rate of POMC gene transcription [40, 52] Although these studies should not be simply transposed to a pathological human condition it should not be totally ruled out that some inescapable POMC gene expression contributes to the unrestrained ACTH secretion especially when the tumor mass becomes important

Both clinical and experimental observations show that situations where maximal ACTH secretion is chronically promted induce an increase in corticotroph cell mass Rare cases of poorly controlled Addisonian patients have apparently developed pituitary enlargement [53–56] A large increase in corticotroph cell area is observed in the anterior pituitary of adrenalectomized rats [57, 58] It is possible that glucocorticoids exert a direct inhibitory action on the growth of corticotroph cells [59], their deprivation being a direct stimulus for growth It is thought however that corticotroph cell growth is driven by the action of CRF Indeed the growth-promoting effect of various hypothalamic neuro-hormones is well documented, like that of growth hormone releasing factor on GH cells for example, which may proceed through the activation of cellular oncogenes [60] Long-term administration of CRF in experimental animals also leads to corticotroph cell hyperplasia [61, 62] and hypertrophy [57]

To explain the growth of a pituitary corticotroph adenoma on these grounds would imply two necessary conditions. that the adenoma be sensitive to the action of CRF, that CRF be present, at least at some time of the development of the adenoma

Pituitary corticotroph adenomas remain sensitive to the stimulatory actions of CRF [63–66] and AVP [67, 68] *in vivo* These actions are used as investigational tools to target the pituitary origin of ACTH oversecretion *In vitro* studies have largely confirmed that the tumoral cells are the direct target of these secretagogs [25–29, 69] A synergystic effect of AVP has also been observed [28] Quantitatively the responses of the tumor cells have rarely been compared to that of normal human corticotroph cells A single study reported that 9 of 16 such tumors had identical sensitivity to CRF as the paired non-adenomatous tissue, and 7 a lower sensitivity [27] In addition to its action on the adenylate cyclase CRF has been shown to modulate action potential firing and to increase intracellular calcium on cultured cells obtained from human corticotroph adenomas [70, 71].

It may seem paradoxical to invoke a role for CRF in the growth of a corticotroph adenoma when much evidence suggests that it is suppressed in Cushing's disease This contradiction is only apparent It is conceivable that, although it is not originally responsible, CRF contributes, at least at the beginning of the disease, to the progression of a pituitary tumor resulting from a clonal event primarily responsible for a set-point defect with secondary tumor growth

3. ARE CORTICOTROPH ADENOMAS CLONAL TUMORS?

The clonal origin of various human endocrine tumors has been recognized, based on the study of genetic markers born by the X chromosome in female heterozygous patients Recent techniques using DNA probes directed at various genetic markers (hypoxanthine phosphoribosyl transferase and/or phosphoglycerate kinase) studied the X-inactivation pattern in peripheral and tumoral tissues through the combined DNA digestion with a methylation-specific enzyme and the restriction enzyme giving rise to a restriction fragment length polymorphism They have shown the monoclonal nature of all

non-functioning pituitary tumors [72, 73] Recent studies performed on functioning tumors [74] showed a monoclonal pattern in three of three GH secreting adenomas, four of prolactin (PRL) secreting adenomas, and three of four corticotroph adenomas, the fourth corticotroph adenoma was substantially contaminated by interspersed normal adenohypophyseal tissue which may have induced an apparent polyclonal pattern

Whatever its mechanism, the occurrence of a state of partial resistance to glucocorticoids in a clone of pituitary corticotroph cells could have, theoretically, the following consequences at the beginning the small tumoral clone would secrete only a minor amount of ACTH with no subsequent increase in cortisol Persistent CRF action on "cortisol deprived" clonal cells would constitute an ideal stimulatory condition for their further growth Thus the adenoma would develop and tumoral ACTH would progressively override non-tumoral ACTH with subsequent increased cortisol production and ultimate extinction of hypothalamic CRF the full-blown expression of Cushing's disease would be attained This scheme could be extended to the situation where bilateral total adrenalectomy would constitute a further stimulus for tumor progression by restoring normal cortisol (exogenously administered) and eventually CRF two conditions that would concur again to stimulate the growth of the tumor, possibly leading to Nelson's syndrome The setpoint defect and the tumor growth potential would be linked This scheme explains how CRF could have a transient role in the progression of the adenoma It shows how at some time a pituitary might contain both an adenoma and still normal corticotroph cells as has been occasionally seen on histological examination [75–78]

REFERENCES

1 De Keyzer Y, Bertagna X, Lenne F, Girard F, Luton J P and Kahn A Altered proopiomelanocortin gene expression in ACTH producing non-pituitary tumors *J Clin Invest* **76** (1985) 1892–1898

2 Suda T, Tozawa F, Yamada M, Ushiyama T, Tomori N, Sumitomo T, Nakagami Y, Demura H and Shizume K Effects of corticotropin-releasing hormone and dexamethasone on proopiomelanocortin messenger RNA level in human corticotroph adenoma cells *in vitro* *J Clin Invest* **82** (1988) 110–114

3 Clark A J L, Lavender P M, Besser G M and Rees L H Pro-opiomelanocortin mRNA size heterogeneity in ACTH-dependent Cushing's syndrome *J Molec Endocr* **2** (1988) 3–9

4 Nayaya T, Seo H, Kuwayama A, Sakurai T, Tsukamoto N, Nakane T, Sugita K and Matsui N Pro-opiomelanocortin gene expression in silent corticotroph-cell adenoma and Cushing's disease *J Neurosurg* **72** (1990) 262–267

5 De Keyzer Y, Bertagna X, Luton J P and Kahn A Variable modes of proopiomelanocortin gene transcription in human tumors *Molec Endocr* **3** (1989) 215–223

6 Bertagna X, Seurin D, Pique L, Luton J P, Bricaire H and Girard F Peptides related to the NH2 terminal end of pro-opiocortin in man *J Clin Endocr Metab* **56** (1983) 489–495

7 Bertagna X, Camus F, Lenne F, Girard F and Luton J P Human joining peptide a proopiomelanocortin product secreted as a homodimer *Molec Endocr* **2** (1988) 1108–1114

8 Vieau D, Massias J F, Girard F, Luton J P and Bertagna X Corticotrophin-like intermediary lobe peptide as a marker of alternate pro-opiomelanocortin processing in ACTH-producing non-pituitary tumours *Clin Endocr* **31** (1989) 691–700

9 Scott A P and Lowry P J Adrenocorticotrophic and melanocyte stimulating peptides in the human pituitary *Biochem J* **139** (1974) 593–602

10 Tanaka N, Abe K, Miyakawa S, Ohnami S, Tanaka M and Takeuchi T Analysis of human pituitary and tumor adrenocorticotropin using isoelectic focusing *J Clin Endocr Metab* **48** (1979) 559–565

11 Ratter S J, Gillies G, Hope H, Hale A C, Grossman A, Gaillard R, Edwards C R W and Rees L H Pro-opiocortin related peptides in human pituitary and ectopic ACTH secreting tumors *Clin Endocr* **18** (1983) 211–218

12 Shibasaki T, Masui H, Sato G, Ling N and Guillemin R Secretion pattern of pro-opiomelanocortin-derived peptides by a pituitary adenoma from a patient with Cushing's disease *J Clin Endocr Metab* **52** (1981) 350

13 Bertagna X, Luton J P, Binoux M, Bricaire H and Girard F Characterization of lipotropin-, corticotropin-, and β-endorphin-immunoreactive materials secreted *in vitro* by a human pituitary adenoma responsible for a case of Nelson's syndrome *J Clin Endocr Metab* **49** (1979) 527–532

14 Suda T, Abe Y, Demura H, Demura R, Shizume K, Tamahashi N and Sasano N ACTH, β LPH and β endorphin in pituitary adenomas of the patients with Cushing's disease activation of β-LPH conversion to β-endorphin *J Clin Endocr Metab* **49** (1979) 475–477

15 Raff H and Findling J W A new immunoradiometric assay for corticotropin evaluated in normal subjects and patients with Cushing's syndrome *Clin Chem* **35** (1989) 596–600

16 Fuller P J, Lim A T W, Barlow J W, White E L, Khalid B A, Copolov D L, Lolait S, Funder J W and Stockrigt J R A pituitary tumor producing high molecular weight adrenocorticotropin-related peptides clinical and cell culture studies *J Clin Endocr Metab* **58** (1984) 134–142

17 Reincke M, Allolio B, Saeger W, Kaulen D and Winkelmann W A pituitary adenoma secreting high molecular weight adrenocorticotropin without evidence of Cushing's disease *J Clin Endocr Metab* **65** (1987) 1296–1300

18 Hale A C, Millar J B, Ratter S J, Pickard J D, Doniach I and Rees L H A case of pituitary dependent Cushing's disease with clinical and biochemical features of the ectopic ACTH syndrome *Clin Endocr* **22** (1985) 479–488

19 Bertagna X, Stone W J, Nicholson W E, Mount C D and Orth D N Simultaneous assay of immunoreactive β-lipotropin, gamma-lipotropin, and β-endorphin in plasma of normal human subjects, patients with

ACTH/LPH hypersecretory syndromes, and patients undergoing chronic hemodialysis *J Clin Invest* **67** (1981) 124–133
20 Brown E M, Le Boff M S, Detting M, Posillico J T and Chen C Secretory control in normal and abnormal parathyroid tissue *Recent Prog Horm Res* **43** (1987) 337–382
21 Liddle G W Tests of pituitary-adrenal suppressibility in the diagnosis of Cushing's syndrome *J Clin Endocr Metab* **20** (1960) 1539–1560
22 Wolfsen A R and Odell W D The dose–response relationship of ACTH and cortisol in Cushing's disease *Clin Endocr* **12** (1980) 557–568
23 Pardes E M, de Yampey J W, Soto R J, Moses D F and De Nicola A F A correlative study between glucocorticoid receptor levels in human mononuclear leukocytes and biochemical data in Cushing's disease *Acta Endocr* **120** (1989) 55–61
24 Kontula K, Pelkonen R, Andersson L and Sivula A Glucocorticoid receptors in adrenocorticoid disorders *J Clin Endocr Metab* **51** (1980) 654–657
25 Grino M, Boudouresque F, Conte-Devolx B, Gunz G, Grisoli F, Oliver C and Jaquet P *In vitro* corticotropin-releasing hormone (CRH) stimulation of adrenocorticotropin release from corticotroph adenoma cells effect of prolonged exposure to CRH and its interaction with cortisol *J Clin Endocr Metab* **66** (1988) 770–775
26 Oosterom R, Verleun T, Vitterlinden P, Hackeng W H L, Burbe J P H, Wiegant V M and Lamberts S W J ACTH and β-endorphin secretion by three corticotropic adenomas in culture Effect of culture time, dexamethasone, vasopressin and synthetic corticotropin-releasing factor *Acta Endocr* **106** (1984) 21–29
27 Suda T, Tomori N, Tozawa F, Demura H and Shizume K Effects of corticotropin-releasing factor and other materials on adrenocorticotropin secretion from pituitary glands of patients with Cushing's disease *in vitro J Clin Endocr Metab* **59** (1984) 840–845
28 White M C, Adams E F, Loizou M, Mashiter K and Fahlbush R Ovine corticotropin-releasing factor stimulates ACTH release from human corticotrophinoma cells in culture interaction with hydrocortisone and arginine vasopressin *Clin Endocr* **23** (1985) 295–302
29 Mashiter K, Adams E F, Gillies G, Van Noorden S and Ratter S Adrenocorticotropin and lipotropin secretion by dispersed cell cultures of a human corticotropic adenoma effect of hypothalamic extract, arginine vasopressin, hydrocortisone and serotonin *J Clin Endocr Metab* **51** (1980) 566
30 Gillies G, Ratter S, Grossman A, Gaillard R, Lowry P J, Besser G M and Rees L H Secretion of ACTH, LPH, and β-endorphin from human pituitary tumors *in vitro Clin Endocr* **13** (1980) 197–205
31 Lamberts S W J, Bons E G and Vitterlinden P Studies on the glucocorticoid-receptor blocking action of RU 38486 in cultured ACTH-secreting human pituitary tumour cells and normal rat pituitary cells *Acta Endocr* **109** (1985) 64–69
32 Lane L, Oldfield E and Brandon D Normal human glucocorticoid receptor gene in pituitary adenomas *The Endocrine Society—71st annual meeting*, Seattle **1374** (1989) 366
33 Hurley D M, Accili D, Vamvakopoulos N, Stratakis C, Taylor S I and Chrousos G P Mutation of the human glucocorticoid receptor gene in familial glucocorticoid resistance *The Endocrine Society—72nd annual meeting*, Atlanta (1990) Abstract 1040 284
34 Qi M, Hamilton B J and DeFranco D v-Mos oncoproteins affect the nuclear retention and reutilization of glucocorticoid receptors *Molec Endocr* **3** (1989)
35 Jaggi R, Salmons B, Muellener D and Groner B The v-Mos and Ha-ras oncogene expression represses glucocorticoid hormone-dependent transcription from the mouse mammary tumour virus LTR *EMBO Jl* **5** (1986) 2609–2616
36 Kahn A Effets antiprolifératif et anti-inflammatoire des glucocorticoides par inhibition des complexes Jun-Fos *Med Sci* **6** (1990) 1003–1005
37 Yan-Yen H F, Chambard J C, Sun Y L, Smeal T, Schmidt T J, Drouin J and Karin M Transcriptional interference between c-Jun and glucocorticoid receptor mutual inhibition of DNA binding due to direct protein–protein interaction *Cell* **62** (1990) 1205–1215
38 Bilezikjian L M, Blount A L and Vale W W The cellular actions of vasopressin on corticotrophs of the anterior pituitary resistance to glucocorticoid action *Molec Endocr* **1** (1987) 451–458
39 Vale W, Vaughan J, Smith M, Yamamoto G, Rivier J and Rivier C Effects of syhthetic ovine corticotropin-releasing factor, glucocorticoids, catecholamines, neuro-hypophysial peptides, and other substances on cultured corticotropic cells *Endocrinology* **113** (1983) 1121–1131
40 Gagner J P and Drouin J Opposite regulation of pro-opiomelanocortin gene transcription by glucocorticoids and CRH *Molec Cell Endocr* **40** (1985) 25–32
41 Sheppard K E, Roberts J L and Blum M Down regulation of glucocorticoid receptor by CRF in AtT20 cells is via an adenylate cyclase dependent mechanism *The Endocrine Society—72nd annual meeting*, Atlanta (1990) Abstr 790 222
42 Vallar L, Spada A and Giannattasio G Altered G and adenylate cyclase activity in human GH-secreting pituitary adenomas *Nature* **330** (1987) 566–568
43 Landis C A, Masters S B, Spada A, Pace A M, Bourne H R and Vallar L GTPase inhibiting mutations activate the alpha chain of G and stimulate adenylyl cyclase in human pituitary tumours *Nature* **340** (1989) 692–696
44 Lyons J, Landis C A and Harsh G Two G protein oncogenes in human endocrine tumors *Science* **249** (1990) 655–659
45 Krieger D T Physiopathology of Cushing's disease *Endocrine Rev* **4** (1983) 22–43
46 Pieters G F F M, Smals A G H, Benraad T J and Kloppenborg P W C Plasma cortisol response to thyrotropin-releasing hormone and luteinizing hormone-releasing hormone in Cushing's disease *J Clin Endocr Metab* **48** (1979) 874–876
47 Krieger D T and Luria M Plasma ACTH and cortisol responses to TRF, vasopressin or hypoglycemia in Cushing's disease and Nelson's syndrome *J Clin Endocr Metab* **44** (1977) 361–368
48 Pieters G F F M, Smals A G H, Goverde H J M, Pesman G J, Meyer E and Kloppenborg P W C Adrenocorticotropin and cortisol responsiveness to thyrotropin-releasing hormone discloses two subsets of patients with Cushing's disease *J Clin Endocr Metab* **55** (1982) 1188–1197
49 Ishibashi M and Yamahi T Direct effects of thyrotropin-releasing hormone, cyproheptadine, and dopamine on adrenocorticotropin secretion from human corticotroph adenoma cells *in vitro J Clin Invest* **68** (1981) 1018–1027
50 Oki S, Nakai Y, Nakao K and Imura H Plasma β-endorphin responses to somatostatin, thyrotropin-releasing hormone, or vasopressin in Nelson's syndrome *J Clin Endocr Metab* **50** (1980) 194
51 Houben H and Denef C Regulatory peptides produced in the anterior pituitary *Trends Endocr Metab*

52 Eberwine J H and Roberts J L Glucocorticoid regulation of proopiomelanocortin gene transcription in the rat pituitary *J Biol Chem* **259** (1984) 2166–2170
53 Scheithauer B W, Kovacs K and Randall R V The pituitary gland in untreated Addison's disease a histological and immunocytologic study of 18 adenohypophyses *Archs Path Lab Med* **107** (1983) 484–487
54 Clayton R, Schrieber V and Burden A C Secondary pituitary hyperplasia in Addison's disease *Lancet* ii (1977) 954–956
55 Krauth B, Muller J, Landolt A M and Von Schulthess F ACTH producing pituitary adenoma in Addison's disease two cases treated by transsphenoidal microsurgery *Acta Endocr* **99** (1982) 357–363
56 Toshihiko Y, Kensaku S, Masaaki A, Hajime N, Ken Ichi K and Hiroschi I Probable ACTH secreting pituitary tumor in association with Addison's disease *Acta Endocr* **110** (1985) 36–41
57 Westlund K N, Aguilera G and Childs G V Quantification of morphological changes in pituitary corticotropes produced by *in vivo* corticotropin-releasing factor stimulation and adrenalectomy *Endocrinology* **116** (1985) 439–445
58 Siperstein E R and Miller K G Hypertrophy of the ACTH-producing cell following adrenalectomy a quantitative electron microscopic study *Endocrinology* **93** (1973) 1257–1268
59 Resetic J, Reiner Z, Ludecke D, Riznar-Resetic V and Sekso M The effects of cortisol, 11-epicortisol, and lysine vasopressin on DNA and RNA synthesis in isolated human adrenocorticotropic hormone-secreting pituitary tumor cells *Steroids* **55** (1990) 98–100
60 Billestrup N, Mitchell R L, Vale W and Verma I Growth hormone-releasing factor induces c-fos expression in cultured primary pituitary cells *Molec Endocr* **1** (1987) 300–305
61 Gertz B J, Contreras L N, McComb D J, Kovacs K, Tyrrell J B and Dallman M F Chronic administration of corticotropin releasing factor increases pituitary corticotroph number *Endocrinology* **120** (1987) 381–388
62 Horacek M J, Campbell G T and Blake C A Effects of corticotrophin-releasing hormone on corticotrophs in anterior pituitary gland allografts in hypophysectomized, orchidectomized hamsters *Cell Tissue Res* **258** (1989) 65–68
63 Orth D N, De Bold C R, De Cherney G S, Jackson R V, Alexander A N, Rivier J, Rivier C, Spiess J and Vale W Pituitary microadenomas causing Cushing's disease respond to corticotrophin-releasing factor *J Clin Endocr Metab* **55** (1982) 1017–1019
64 Pieters G F F M, Hermus A R M M, Smals A G H, Bartelink A K M, Benraad T G and Kloppenborg P W C Responsiveness of the hypophyseal-adrenocortical axis to corticotropin releasing factor in pituitary-dependent Cushing's disease *J Clin Endocr Metab* **57** (1983) 513–516
65 Muller O A, Dorr H G, Hagen B, Stalla G K and Von Werder K Corticotropin releasing factor stimulation test in normal controls and patients with disturbances of the hypothalamo-pituitary-adrenal axis *Klin Wochenschr* **60** (1982) 1485–1491
66 Chrousos G P, Schulte J M, Oldfield E H, Gold P W, Cutler G B Jr and Loriaux D L The corticotropin-releasing factor stimulation test An aid in the evaluation of patients with Cushing's syndrome *New Engl J Med* **310** (1984) 622–626
67 Raux M C, Binoux M, Luton J P, Pham-Huu-Trung M T and Girard F Studies of ACTH secretion control in 116 cases of Cushing's syndrome *J Clin Endocr Metab* **40** (1975) 186–197
68 Binoux M, Gourmelen-Combourieu M, Luton J P, Pham Huu Trung M T and Girard F Etude de l'ACTH plasmatique au cours de 100 épreuves à la lysine vasopressine chez l'homme *Acta Endocr* **68** (1971) 1–30
69 Suda T, Tozawa F, Mouri T, Sasaki A, Shibasaki T, Demura H and Shizume K Effect of cyproheptadine, reserpine and synthetic corticotropin-releasing factor on pituitary glands from patients with Cushing's disease *J Clin Endocr Metab* **56** (1983) 1094–1099
70 Spada A, Reza-Elahi F, Lania A, Bassetti M and Atti E Inhibition of basal and corticotropin-releasing hormone-stimulated adenylate cyclase activity and cytosolic Ca2+ levels by somatostatin in human corticotropin-secreting pituitary adenomas *J Clin Endocr Metab* **70** (1990) 1262–1268
71 Mollard P, Vacher P, Guerin J, Rogawski M A and Dufy B Electrical properties of cultured human adrenocorticotropin-secreting adenoma cells effects of high K+, corticotropin-releasing factor and angiotensin II *Endocrinology* **121** (1987) 395–405
72 Klibanski A Editorial further evidence for a somatic mutation theory in the pathogenesis of human pituitary tumors *J Clin Endocr Metab* **71** (1990) 1415A–1415C
73 Alexander J M, Biller B M K, Bikkal H, Arnold A and Klibanski A Clinically non-functioning pituitary tumors are monoclonal in origin *J Clin Invest* **86** (1990) 336–340
74 Herman V, Fagin J, Gonsky R, Kovacs K and Melmed S Clonal origin of pituitary adenomas *J Clin Endocr Metab* **71** (1990) 1427–1433
75 Lamberts S W J, Stefanko S Z, De Lange S, Fermin M, Van der Vijver J C M, Weber R F A and de Jong F H Failure of clinical remission after transsphenoidal removal of a microadenoma in a patient with Cushing's disease, multiple hyperplastic and adenomatous cell nests in surrounding pituitary tissue *J Clin Endocr Metab* **50** (1980) 793–798
76 Ludecke D, Kautzky R, Saeger W and Schrader D Selective removal of hypersecreting pituitary adenomas *Acta Neurochir* **35** (1976) 27–42
77 Saeger W, Ludecke D L and Geisler F The anterior lobe in Cushing's disease/syndrome In *ACTH, Cushing's Syndrome, and Other Hypercortisolemic States* (Edited by D L Ludecke, G P Chrousos and G Tolis) Raven Press, New York (1990) pp 147–156
78 Cook D M and McCarthy P J Failure of hypophysectomy to correct pituitary-dependent Cushing's disease in two patients *Archs Int Med* **148** (1988) 2497–2500