

Medical Therapy for Cushing's Disease – Past and Future Modes of Treatment

a report by

Krystallenia I Alexandraki¹ and Ashley B Grossman²

1. Specialist in Endocrinology; 2. Professor of Neuroendocrinology, and Consultant Physician, Department of Endocrinology, St Bartholomew's Hospital, London

Cushing's disease is the most frequent cause of endogenous hypercortisolaemia.¹ As in other cases of Cushing's syndrome, the goals of treatment are the normalisation of cortisol levels with a reversal of clinical symptoms in order to avoid the long-term consequences of hypercortisolism. Surgical removal of the adenoma is the current first-line therapeutic approach, which may be followed by radiotherapy in cases of surgical failure.² When these treatments have failed, drugs represent the next step in therapy, although they do not play a role in primary therapy as in other types of secretory pituitary tumours such as prolactinomas or acromegaly. However, their utility is reflected by the necessity to treat glucocorticoid excess in order to reverse the metabolic consequences and poor healing in severely affected patients, and this may be required before surgery. In cases where surgical treatment fails, drugs are an alternative as monotherapy or in addition to radiotherapy while awaiting its delayed effects. Finally, medical treatment may be considered in patients who cannot be submitted to surgical procedures because of co-morbidities, or who are unwilling to receive other types of treatment.

The ideal drug for Cushing's disease that targets the pituitary has not been found. Variable compounds with neuromodulatory properties, including dopamine agonists and somatostatin analogues, gamma-aminobutyric acid (GABA) agonists, serotonin antagonists and different nuclear hormone receptor ligands involved in hypothalamo-pituitary regulation (thiazolidinediones and retinoic acid), have been tested. On the other hand, compounds that target glucocorticoid synthesis (adrenal secretion inhibitors or adrenolytic drugs, such as aminoglutethimide, metyrapone,

ketoconazole, etomidate, mitotane or trilostane) or function (glucocorticoid antagonists: mifepristone) have so far been broadly used to control the deleterious effects of the hypercortisolaemic state. Those pharmaceutical agents will be summarised in this article.

Neuromodulatory Compounds

Somatostatin Analogues

Somatostatin (SST) is a neuropeptide whose actions are mediated through five different membrane-bound receptors (SSTR1–5). SSTR1, 2, 4 and 5 inhibit cell proliferation via a phosphotyrosine-phosphatase-dependent pathway and interact with the mitogen-activated protein kinase pathway,^{3,4} although recent data suggest an additional interaction with a serine-threonine phosphatase.⁵ SSTR3 is cytotoxic and causes cell death or apoptosis through a phosphotyrosine-phosphatase-dependent mechanism and activation of the p53 and Bax proteins.⁴ Although the heterogeneity of the studies performed and the differences in methodology have resulted in some contradictory findings, human corticotroph adenomas express multiple SSTR subtypes, with 1, 2 and 5 being the most frequently found; both SSTR2 and 5 seem to be implicated in the regulation of adrenocorticotropin (ACTH) release, but SSTR5 is considered to be the predominant receptor.^{6–10} In *in vitro* studies of animal-derived and human corticotroph adenoma cell lines, native SST and octreotide, a predominantly SSTR2-selective ligand having moderate affinity for SSTR5, inhibit basal and stimulated adrenocorticotrophic hormone (ACTH) secretion.^{7–9,11} However, *in vitro* studies support the fact that this inhibition is present when the corticotroph cells have been cultured in corticosteroid-free medium.^{12–16} In patients with Cushing's disease most experience has been gained with octreotide; however, this has been proved to be virtually ineffective.^{17–20}

Pasireotide or SOM-230 (Novartis, Basel, Switzerland) is a new multiligand SST analogue that has high binding affinity to SSTR5 and 1, 2 and 3 subtypes.²¹ It was found to inhibit basal and stimulated ACTH release from human ACTH-secreting pituitary adenomas and the murine corticotroph tumour cell line AtT-20 *in vitro*, without inhibiting AtT20 cell proliferation, nor inducing apoptosis or inhibiting pro-opiomelanocortin synthesis, implying an action through a possible blockade of ACTH release or an increased breakdown of ACTH.^{7,22,23} The functional activity of pasireotide compared with octreotide has been found to be 30-, 11- and 158-fold higher on SSTR1, 3 and 5, respectively, and approximately seven-fold lower on SSTR2,²¹ being more potent compared with octreotide in inhibiting basal ACTH release.⁷ In *in vitro* studies in both human ACTH-secreting adenomas and the murine AtT20 cell line, pasireotide suppressed ACTH secretion and corticotrophin-releasing hormone (CRH)-induced ACTH release more than octreotide.^{7,22} Pre-incubation with dexamethasone did not affect the ability of pasireotide to inhibit CRH-induced ACTH release, while the suppressive action of octreotide was virtually lost.^{7,22} Furthermore,



Krystallenia Alexandraki is a Specialist in Endocrinology in the Department of Endocrinology at St Bartholomew's Hospital in London. Her research interests include pituitary diseases, neuroendocrine tumours, polycystic ovarian syndrome and inflammation markers. She is the author of more than 130 original articles, review articles, articles in proceedings, abstracts in journals or books and chapters in editions, and is a member of several scientific associations. Dr Alexandraki has a PhD in endocrinology, an MSc in clinical biochemistry and an MSc in health management.

E: alexandrakik@endo.gr



Ashley Grossman is a Professor of Neuroendocrinology and a Consultant Physician in the Department of Endocrinology at St Bartholomew's Hospital, London. He is Past President of the European Neuroendocrine Association (ENA), a Fellow of the Academy of Medical Sciences, Past Editor of *Clinical Endocrinology* and on the Editorial Board of the major textbook *De Groot and Jameson's Endocrinology*. Dr Grossman has published nearly 600 research papers and reviews and has a

particular interest in pituitary and hypothalamic tumours, endocrine oncology and the molecular pathogenesis of neuroendocrine tumours.

E: A.B.Grossman@qmul.ac.uk

SSTR2A and 2B (but not SSTR5) messenger RNA (mRNA) levels were significantly suppressed after 24 and 48 hours of dexamethasone treatment.⁷ These findings imply a differential impact of glucocorticoids on the expression of the different SSTRs,^{17,19–21,24} with SSTR2 being downregulated and SSTR5 resistant to corticosteroid modulation. Sensitivity to SST inhibition of ACTH secretion is possibly observed only when the physiological feedback regulation of ACTH release by glucocorticoids has been lost;¹⁶ this hypothesis is supported by the absence of any effect on ACTH levels after experimental infusions of natural SST or octreotide in normal individuals,^{25,26} as well as by their suppression in patients with adrenal insufficiency or adrenalectomy.^{17,27–29} A direct effect of glucocorticoids at the transcriptional level or on mRNA stability has also been suggested.³⁰ Recently, pasireotide was shown to significantly suppress cell proliferation as well as ACTH secretion in human corticotroph tumours,²³ but possibly via independent mechanisms since no correlation was found between these effects. Another mechanism for its action might be an indirect influence of growth by decreasing secretion of pituitary hormones and/or growth factors.³¹ Recently, the use of pasireotide in the treatment of Cushing's disease was investigated in a phase II, open-label, single-arm, multicentre pilot study.³² Twenty-nine patients self-administered subcutaneous pasireotide 600µg twice daily for 15 days. Remission of urinary cortisol to within the normal reference range was demonstrated in 17.2% of patients and a reduction in urinary free cortisol levels in 75.8%, along with a reduction in serum cortisol and plasma ACTH levels. These encouraging results suggest that pasireotide may be a promising novel therapy for Cushing's disease with both antisecretory and potential antiproliferative properties; it may also be that inhibiting ACTH release and subsequent cortisol levels via SSTR5 will restore SSTR2 expression, and hence further aid ACTH inhibition.

Dopaminergic Neuromodulators

Dopamine is the predominant catecholamine neurotransmitter in the human central nervous system,³³ acting via its receptors (D1–5). The D2 receptor is expressed in the anterior and intermediate lobe of the pituitary gland,^{34,35} showing variable and heterogeneous expression in 89% of all types of pituitary tumours.³⁶ It has also been detected in 69% of silent or functioning ACTH-secreting pituitary tumours in one study³⁶ and in more than 75% of such tumours in another;³⁷ its presence has been correlated with the inhibitory effect of dopamine agonists on ACTH secretion *in vitro*.³⁷ However, no specific binding of a dopamine agonist was demonstrated in some corticotroph pituitary tumours,³⁸ and the D2 receptor was not demonstrated by ¹²³I-epidepride and single-photon emission computed tomography (CT) imaging in Cushing's disease or Nelson's syndrome.³⁹

Dopaminergic modulation of ACTH secretion has been suggested to occur via regulation of hypothalamic CRH release in addition to direct inhibition of ACTH secretion by the corticotrophs.^{40,41} The dopamine agonist bromocriptine suppressed ACTH secretion from cultured human pituitary tumour cells⁴² and induced apoptosis in AtT-20 cells.⁴³ In Cushing's disease, bromocriptine treatment has shown variable results and its therapeutic potential is considered very limited.^{40,41,44–46} In different studies the effectiveness of bromocriptine has varied between 0 and 50%, with normalisation of urinary and/or plasma cortisol in up to 40% of patients in various case reports and small series,⁴⁰ but probably far fewer than 10% of unselected patients respond to this therapy.^{40,46,47} Bromocriptine has also been considered in cases of cyclical Cushing's disease,⁴⁸ and its beneficial effect has been suggested to be a diagnostic tool for this peculiar disorder. Cabergoline has also been investigated and reported to inhibit ACTH

secretion *in vitro* in cases with D2-receptor-positive cells.³⁷ After three months of therapy with cabergoline at doses of 2.5–3.5mg/week, a decrease of greater than 50% in daily urinary-free cortisol level and a normalisation of cortisol production were observed in 60 and 40% of patients, respectively.³⁷ In addition, cabergoline has been successfully used in a case of pituitary macroadenoma-secreting aberrant ACTH⁴⁹ and in small series⁵⁰ showing tumour shrinkage and a decrease in cortisol levels. On the other hand, a direct effect on peripheral cortisol secretion has not been identified.⁵¹ In a more recent study, it was demonstrated that cabergoline treatment at doses of 1–7mg/week were effective in controlling cortisol

Pasireotide may be a promising novel therapy for Cushing's disease with both antisecretory and potential antiproliferative properties.

secretion for at least one to two years in more than one-third of patients with CD after failure of surgical treatment.⁵² The expression of the D2 receptor in 80% of corticotroph pituitary tumours and the effectiveness of cabergoline treatment in the normalisation of cortisol secretion in patients with Cushing's disease justifies consideration of the use of cabergoline for controlling this disorder.³⁷ However, the long-term consequences of dopamine agonists (specifically cabergoline)^{53,54} and their value, as well as their combination with SST receptor ligands such as pasireotide,^{2,55} require further investigation.

Other Neuromodulatory Compounds and Ligands of Different Nuclear Hormone Receptors

Cyproheptadine is a histamine and serotonin antagonist with poor selectivity for receptor subtypes that also possesses anticholinergic activity.⁵⁶ It has been assumed that direct serotonergic central nervous system control modulates hypothalamic factors that promote ACTH secretion⁵⁷ or that it exerts a direct inhibitory effect on CRH and vasopressin secretion from the hypothalamus.^{58,59} In clinical studies, the response rate in patients treated with cyproheptadine or the similar metergoline has been poor, as has been the case with the more selective serotonin antagonists ritanserin and ketanserin;^{57,60–63} however, more positive results have been reported in cases with resistant microadenoma⁶⁴ or in cases with no evidence of adenoma on imaging that have been suggested to represent 'hypothalamic' Cushing's disease.⁵⁹ It was predicted that up to 70% of patients with Cushing's disease would respond to cyproheptadine treatment,⁶⁰ but its variability in clinical effects, its sedative side effects and the increase in the appetite of patients observed limit its use.^{56,62} Despite the fact that these compounds may have a direct effect on signalling pathways in the corticotroph cell independent of any effect on neurotransmitters, this form of therapy is limited by its adverse effects having no role in the modern management of Cushing's disease.

Sodium valproate (valproic acid) inhibits β -aminobutyric acid aminotransferase. In patients with epilepsy it was shown that it decreases ACTH levels, possibly inhibiting CRH and hence ACTH release.^{65,66} Placebo-controlled studies of the agent did not support its use as monotherapy,⁶⁷ although its combination with inhibitors of cortisol secretion appeared to be beneficial.⁶⁸ It is possible that it may alter

cortisol metabolism, indirectly affecting ACTH release, but it does not seem to be of value in the therapy of corticotroph tumours.

Retinoic acid is an effective compound in reducing ACTH plasma levels and tumour size in animal models,^{69,70} but no human clinical trial has yet been performed. In rodent models it decreases corticotroph secretion and proliferation,⁶⁹ as well as in a canine model of Cushing's disease, producing a significant decrease in ACTH and cortisol levels, amelioration of clinical signs, prolongation of survival and pituitary tumour shrinkage without obvious side effects.⁷⁰ Retinoic acid is a ligand for Nur77/Nurr1 and possibly

Cabergoline has been successfully used in a case of pituitary macroadenoma-secreting aberrant ACTH and in small series showing tumour shrinkage and a decrease in cortisol levels.

of activator protein 1 (AP-1), which are positive transcriptional regulators of the ACTH gene in ACTH-secreting cells.^{69,71,72} Proopiomelanocortin (POMC) stimulation by CRH is mediated by Nur77 and may also involve AP-1, and is inhibited by retinoic acid; in addition, a reduction of the CRH-stimulated POMC transcription was produced by mutation of the NurRE or AP-1-binding sites.^{69,71,73} Endogenous production of ACTH was reduced by retinoic acid in AtT-20 tumour cells but not in normal pituitary cells, suggesting that retinoic acid is not part of the normal physiological feedback mechanism.⁶⁹ Genes that have been found to be differentially expressed in pituitary tumours might also be involved in the differential action of retinoic acid.^{72,74,75} In addition, retinoic acid inhibits ACTH- and corticosterone-secreting cell proliferation, and it is possible that other *in vivo* pathways independent of AP-1 or Nur77/Nurr1 contribute to this effect.⁶⁹ Additional beneficial effects include the reduction of adrenal hyperplasia by direct inhibition of adrenal cell proliferation, reversal of skin atrophy and immunostimulation.⁷⁶

Peroxisome proliferator-activated receptor-gamma (PPAR- γ) agonists (rosiglitazone and pioglitazone) have been suggested to be promising new agents specifically targeting pituitary tumours, based on *in vitro* and rodent models.^{77,78} However, recent small-scale clinical trials with rosiglitazone and pioglitazone have not shown any clear therapeutic effect in Cushing's disease.^{79,80} PPAR- γ belongs to the family of nuclear hormone receptors⁸¹ and ligands for the nuclear receptor and had been shown to induce cell-cycle arrest and apoptosis in corticotroph tumour cells and tumour growth arrest *in vivo* in a mouse model, and to inhibit ACTH and corticosterone secretion from tumour cells.⁷⁸ PPAR- γ expression has been described in both normal and adenomatous pituitary tissue.^{77,78,82,83} A more recent study using more sensitive methodology revealed that PPAR- γ receptor is only poorly expressed in human pituitary tissue; the authors were unable to detect any specific abnormality of PPAR- γ expression in corticotroph tumours, describing poor immunocytochemical expression in both normal pituitary and pituitary adenomas with only weak cytoplasmic staining.⁸⁴ Furthermore, rosiglitazone showed antiproliferative effects only at very high doses, and these were not blocked by a specific PPAR- γ antagonist.⁸⁴ Combining these results with the lack of a consistent effect on ACTH and cortisol levels in controls⁸⁵ or patients with Cushing's disease after

short- or long-term administration,^{79,80,86} it seems that the treatment of pituitary tumours with PPAR- γ agonists is unlikely to be worth pursuing, and any effect of rosiglitazone or pioglitazone is also unlikely to involve the PPAR- γ receptor.⁸⁴

Inhibition of Cortisol Function

Glucocorticoid Antagonist

Mifepristone (RU 486) is a steroid that binds competitively to the glucocorticoid, androgen and progestin receptors and inhibits the action of the endogenous ligands.^{44,87} Its use in Cushing's disease has been limited.^{87,88} Assessment of responsiveness and dose monitoring is difficult, since the drug interferes with the glucocorticoid negative feedback at the hypothalamo-pituitary level and induces a rise in ACTH and cortisol secretion.⁸⁷ This renders any assessment of response subject to clinical appraisal, while the blockade of cortisol receptors and loss of feedback necessitates frequent imaging,⁸⁹ limiting its use as monotherapy in Cushing's disease.⁹⁰ Interestingly, it has been used in one patient with an ACTH-secreting pituitary macroadenoma after radiation therapy who responded extremely well to high doses (up to 25mg/kg daily) with remission of life-threatening clinical symptomatology.⁸⁸ Potential adverse effects that have been reported in patients treated with high-dose mifepristone for long periods include adrenal insufficiency with difficulty in biochemical confirmation,⁸⁷ as well as severe hypokalaemia attributed to excessive cortisol activation of the mineralocorticoid receptor that required treatment with spironolactone.⁸⁸ Further evaluation of its safety and of follow-up methodology may render this agent a more attractive option, and clinical trials in the ectopic ACTH syndrome are currently under way.

Inhibition of Cortisol Secretion

These compounds decrease cortisol levels by direct inhibition of steroidogenesis at one or more enzymatic steps, being highly effective in treating hypercortisolism but not the underlying tumour, and they do not restore normal HPA secretory dynamics. Ketoconazole and metyrapone are the most frequently used drugs, and appear to be more effective and better tolerated than aminoglutethimide.⁹¹⁻¹⁰⁰ All of these drugs require escalation after an initial low dose to minimise their side effects, and frequent monitoring is required to achieve an acceptable clinical and biochemical profile. The inhibition can be partial or complete, requiring a 'block-and-replacement' scheme. In either case, patients should be well instructed about adrenal insufficiency symptoms or the recurrence of hypercortisolism symptoms. In addition, there may be a requirement to increase the dose over time to maintain the desirable cortisol levels, since corticotroph tumours have a higher than normal set-point for cortisol negative feedback, and ACTH secretion may increase in parallel with the fall in cortisol. Occasional patients rarely remain in remission after their discontinuation when given as monotherapy.¹⁰¹⁻¹⁰⁵

Metyrapone blocks 11 β -hydroxylase and has a rapid onset of action.⁴⁰ It may be useful as monotherapy or in combination with other drugs, or following radiotherapy. Good control of cortisol levels is frequently obtained^{68,101,106-109} in both short- and long-term studies.¹⁰⁷⁻¹¹¹ In one case report a 13-year-old boy received a 2g regimen of metyrapone for four years with resolution of cushingoid features and a gain of 23cm in height,¹⁰⁹ while a larger study of 53 patients reported effective short-term mean serum cortisol level control (<400nmol/l) in 75% of patients with effective long-term control in 83% of the 24 patients (mean 2,250mg/day, median treatment duration 27 months) following pituitary irradiation.¹⁰¹ The rate of the positive results

from these studies is related to each study 'cut-off' values for 'cortisol control';⁸⁹ however, it is generally accepted that metyrapone is a useful adjunctive treatment for Cushing's disease.¹⁰² Interestingly, the block of adrenal steroidogenesis cannot be overcome even in cases with persistent increase of ACTH secretion along with the normalisation of cortisol levels.^{108–110} Metyrapone is given at an initial daily dose of 0.5–1g, in three to four divided doses daily, and may be increased every few days to a maximal daily dose of 6–8g.^{44,112,113} It has been used successfully pre-operatively, lowering mean cortisol levels to less than 400nmol/l with dose titration;¹⁰¹ the more recent target is a mean serum cortisol level below 300nmol/l.¹¹⁴ Since metyrapone results in increased ACTH secretion and thereby increased androgenic precursors with subsequent acne and hirsutism, this is a common cause of treatment discontinuation.^{101,108,111} The appearance of hypertension, hypokalaemia and oedema due to mineralocorticoid precursors are not serious side effects since cortisol levels are also reduced by the treatment.^{101,112,115} Other side effects include dizziness and gastrointestinal upset,^{89,113} although it is possible that some of these are in fact due to inadvertent induction of adrenocortical insufficiency.

Ketoconazole is an imidazole derivative that acts at a number of sites. It inhibits cytochrome P450 enzymes, with strongest effect on 17,20-lyase, and side-chain cleavage, 11 β -hydroxylase and 17 β -hydroxylase, but there is disagreement for the latter role.^{99,116,117} Interestingly, extra-adrenal actions have been reported. Ketoconazole caused an acute *in vitro* decrease in basal and CRH-stimulated ACTH secretion by corticotroph tumour cells obtained from two patients with Nelson's syndrome,¹¹⁸ showing similar effects on rat anterior pituitary cells *in vitro* in one study¹¹⁹ but not in another.⁹³ The fact that ACTH concentrations increase during long-term treatment with ketoconazole suggests that its major effect is on the adrenal cortex rather than the corticotroph cells. In addition, at high concentrations it has been shown to be an antagonist of the glucocorticoid receptor in cultured hepatoma cells¹²⁰ and to bind to glucocorticoid receptors in cytosolic preparations of human mononuclear cells.¹²¹

A meta-analysis of 82 patients with presumed Cushing's disease showed that monotherapy with ketoconazole at daily doses of 400–1,600mg effectively reduced serum cortisol levels by 70% (range 25–93%).¹²² It has also been administered as adjuvant therapy in conjunction with radiation therapy⁹⁸ or in combination with other drugs such as octreotide, with equally satisfactory results.¹²³ However, these trials are based on few patients or are biased by the co-administration of other treatments. Interestingly, when given to five women with presumed Cushing's disease for three months, urinary cortisol excretion fell and plasma ACTH levels decreased by 30–75%.¹²⁴ In another study, basal ACTH concentrations were increased compared with the pre-treatment values of four to six patients with presumed Cushing's disease, and CRH-stimulated ACTH concentrations were increased in all, suggesting that ketoconazole may not suppress corticotroph tumours *in vivo*⁹² or that the elevation is due to a change in feedback.⁸⁹ In the previous meta-analysis ACTH concentrations were increased by an average of 35% in patients in remission and 80% in patients with persistent disease. This limitation can be reversed by using additional adrenal enzyme inhibitors.⁸⁹ Treatment with ketoconazole is usually started at a daily dose of 400mg (divided into two doses) and increased every three or more days to a maximum of 1.2–1.6g daily in three or four divided doses.^{44,89} As gastric acidity is necessary to metabolise it into the active compound, ketoconazole is not an option in achlorhydric patients or those treated with proton pump antagonists unless it is formulated locally in an acidic vehicle.⁴⁴ An idiosyncratic hepatic dyscrasia occurs in about one

in 15,000 cases, which has limited its use in occasional patients, and this may occasionally be fatal;^{125–129} otherwise, it has a relatively benign spectrum of side effects such as gastrointestinal symptoms, gynaecomastia, irregular menses and reversible hepatic dysfunction, which do not necessarily require its discontinuation.⁴⁴ Other noted side effects include decreased libido and impotence, probably owing to its inhibitory effects on 17 β -hydroxylase and 17,20-lyase, which are the main cause of treatment discontinuation in men,¹¹² the teratogenicity that contraindicates its use in pregnancy and its interactions with other medications because of the potent inhibitory effects on cytochrome P450 enzymes (particularly CYP3A4, CYP2C9 and CYP1A2). However, a recent clinical study in 38 patients supported the clinical value of ketoconazole as safe and efficacious treatment in CD, particularly in patients for whom surgery is contraindicated or delayed because of the absence of image of an adenoma on magnetic resonance imaging.¹³⁰

Aminoglutethimide acts at a number of sites.¹³¹ It inhibits side-chain cleavage of cholesterol to pregnenolone and cortisol, oestrogen and aldosterone production.^{132,133} In addition, it inhibits 11 β -hydroxylase, 18-hydroxylase and aromatase.¹³⁴ It is not considered efficacious as monotherapy^{103,135} compared with its action as combination therapy,^{107,111} partly due to the feedback in ACTH, which negates its effect.¹²² It is given at a dose of 500mg daily in divided doses and can be increased by 250–500mg every three to four days to a total daily dose of 2g. In combination with metyrapone, it has been observed to be effective at up to one year at daily doses of 500–750mg together with 2g of metyrapone.¹¹¹ In an original study of 33 patients with Cushing's disease receiving a 250mg dosage three times daily, a clinical and biochemical remission rate of 42% were reported,¹⁰³ but the authors concluded that bilateral adrenalectomy was preferable for continued biochemical remission. Neurological and gastrointestinal side effects and tolerance with continued treatment limit its use, although it has been stated that the presence of a transient morbilliform rash, fever and mild impairment of thyroid function are not reasons to discontinue therapy.^{103,111,131} Other side effects include dizziness, blurred vision, cholestasis and bone marrow suppression.^{44,89} In addition, aminoglutethimide is a strong inducer of several cytochrome P450 enzymes (including CYP1A2 and CYP3A4) with several drug interactions, including a fall in concomitant ketoconazole levels.⁸⁹ Finally, the fact that it increases the metabolism of dexamethasone suggests that hydrocortisone is preferable if steroid replacement is needed.^{131,136} However, we believe that there is little place for this drug in the modern therapy of Cushing's disease.

Endogenous production of ACTH was reduced by retinoic acid in AtT-20 tumour cells but not in normal pituitary cells, suggesting that retinoic acid is not part of the normal physiological feedback mechanism.

Etomidate is an imidazole derivative and, compared with ketoconazole, inhibits 11 β -hydroxylase more potently, showing a similar inhibition of 17 β -hydroxylase but a lesser effect on 17,20 lyase;¹³⁷ in higher concentrations it has an effect on cholesterol side-chain cleavage.^{138,139} Blunting of the cortisol response to ACTH has been reported *in vivo*^{140,141} and inhibition of ACTH-stimulated cortisol secretion from dispersed adrenal

cells *in vitro*,¹⁴² suggesting a direct effect on the adrenal cortex. Studies in animal models adrenal cells and in case reports as well suggest inhibition at several enzymatic steps.^{139,143,144} A non-sedating continuous intravenous dose of 0.3mg/kg/hour effectively reduced serum cortisol within 12 hours in six patients.¹⁴⁵ Etomidate is the only compound active in an intravenous form and could well be an alternative when rapid control of cortisol levels is required and oral therapy cannot be administered,^{146–148} as in cases of critically ill patients with Cushing's disease, including children.^{144,148}

Trilostane blocks 3 β -hydroxysteroid dehydrogenase and is a relatively weak inhibitor of steroidogenesis.¹⁴⁹ Although it has been demonstrated to show beneficial effects in animal models,¹⁵⁰ disappointing results have been reported in human trials and its use is not recommended.¹⁵¹

Mitotane (O,p'DDD) inhibits steroidogenesis at the steps of side-chain cleavage, 11- and 18-hydroxylase and 3 β -hydroxysteroid dehydrogenase.^{44,152–154} It has been considered to be highly effective in the long-term suppression of hypercortisolism in patients with Cushing's disease because of its specific adrenolytic action. If used at high doses (greater than 4g per day) its metabolite binds macromolecules in adrenocortical cell mitochondria, leading to their destruction and cellular necrosis, resulting over time in permanent glucocorticoid and mineralcorticoid replacement therapy.⁴⁴ Consequently, when 12g/day is used as monotherapy, remission has been observed in up to 83% of patients with Cushing's disease, and was sustained after discontinuation in about one-third of them.¹⁵⁵ It has been used moderately effectively as an adjunct to radiation therapy,^{96,156} with clinical and biological remission observed in 81% of 36 patients, with 47% ceasing long-term mitotane therapy.⁹⁶ Four patients had no response, two had a partial response and the other patient died of an invasive pituitary malignancy. Mitotane is often started at 250–500mg nightly with slow escalation of the dose to 4–12g/day,⁸⁹ and it is important to regularly monitor drug levels.¹⁵⁷ The disadvantages of its use include a slow onset of action (weeks or months), a long half-life,¹⁵⁸ significant gastrointestinal and neurological adverse effects, hypercholesterolaemia¹⁵⁹ and less common problems such as gynaecomastia.^{44,89,113} When glucocorticoid and mineralcorticoid replacement therapy begins dexamethasone is an option because of its longer half-life, but its metabolism is induced by mitotane, requiring dose adjustments, whereas the metabolism of cortisol or prednisone is not.¹⁶⁰ However, mitotane induces a large rise in cortisol-binding globulin such that measurement of total cortisol is unreliable, and it is necessary to rely

on plasma ACTH measurement or urinary free cortisol to avoid an adrenocortical crisis. Of note, patients under medical mitotane therapy are at risk of developing Nelson's syndrome and pituitary radiotherapy is an option.⁸⁹ In general, the problems associated with the use of mitotane determine that its use be confined to the minority of patients who are intolerant or not responsive to ketoconazole and/or metyrapone, and who are unsuitable for adrenalectomy.

Conclusions

An effective medical therapy that targets the pituitary adenoma would be a valuable therapeutic option for the management of Cushing's disease; however, currently there is no effective medical therapy that directly and reliably targets the ACTH-secreting pituitary adenoma and thus the underlying cause of the disease. There are encouraging developments such as the somatostatin analogue pasireotide, from a phase II trial, and dopamine agonist cabergoline, from a long-term clinical study. The chimeric somatostatin-dopamine ligand dopastatin (developed to treat other types of pituitary tumour) may also show therapeutic promise in Cushing's disease. It will also be of considerable clinical interest to design therapeutic trials with retinoic acid analogues. Consequently, since few currently available drugs are able to directly inhibit ACTH release and the fact that Cushing's disease is the most frequent cause of endogenous hypercortisolaemia, therapy targeting the adrenal remains the mainstay of therapy. Worldwide experience with both ketoconazole and metyrapone (either alone or in combination) suggests that these agents are the most effective primary treatment for Cushing's disease. However, the presence of hirsutism frequently precludes metyrapone therapy in women, while gynaecomastia or hypogonadism requires caution with the use of ketoconazole therapy in men, and the drug may be hepatotoxic; gastrointestinal symptoms may occur due to both compounds in both sexes. The difficulty in monitoring and the adverse effects of mitotane determine that its use be confined to the minority of patients not responsive or intolerant to ketoconazole and/or metyrapone. The other adrenal inhibitors are now rarely needed; however, etomidate remains an important option when intravenous administration is the only way of treating severely ill patients. Finally, mifepristone has been used in limited cases, but further evaluation of its safety and follow-up methodologies may render this agent a more attractive option. Nonetheless, if patients remain intolerant or incompletely responsive to medical therapy, it is extremely important to remember that bilateral laparoscopic adrenalectomy will cause immediate remission of hypercortisolaemia when all else fails. ■

- Makras P, Toloumis G, Papadogias D, et al., The diagnosis and differential diagnosis of endogenous Cushing's syndrome, *Hormones*, 2006;5:231–50.
- Biller BM, Grossman AB, Stewart PM, et al., Treatment of adrenocorticotropin-dependent Cushing's syndrome: a consensus statement, *J Clin Endocrinol Metab*, 2008;93:2454–62.
- Patel YC, Somatostatin and its receptor family, *Front Neuroendocrinol*, 1999;20:157–98.
- Sharma K, Patel YC, Srikanth CB, C-terminal region of human somatostatin receptor 5 is required for induction of Rb and G1 cell cycle arrest, *Mol Endocrinol*, 1999;13:82–90.
- Grozinsky-Glasberg S, Franchi G, Teng M, et al., Octreotide and the mTOR inhibitor RAD001 (everolimus) block proliferation and interact with the Akt-mTOR-p70S6K pathway in a neuro-endocrine tumour cell line, *Neuroendocrinology*, 2008;87:168–81.
- Nielsen S, Mellemejkjaer S, Rasmussen LM, et al., Expression of somatostatin receptors on human pituitary adenomas *in vivo* and *ex vivo*, *J Endocrinol Invest*, 2001;24:430–37.
- van der Hoek J, Waaaijers M, van Koetsveld PM, et al., Distinct functional properties of native somatostatin receptor subtype 5 compared with subtype 2 in the regulation of ACTH release by corticotroph tumor cells, *Am J Physiol Endocrinol Metab*, 2005;289:E278–87.
- Richardson UI, Schonbrunn A, Inhibition of adrenocorticotropin secretion by somatostatin in pituitary cells in culture, *Endocrinology*, 1981;108:281–90.
- Heisler S, Reisine TD, Hook VV, Axelrod J, Somatostatin inhibits multireceptor stimulation of cyclic AMP formation and corticotropin secretion in mouse pituitary tumor cells, *Proc Natl Acad Sci U S A*, 1982;79:6502–6.
- Viollet C, Vaillend C, Videau C, et al., Involvement of sst2 somatostatin receptor in locomotor, exploratory activity and emotional reactivity in mice, *Eur J Neurosci*, 2000;12:3761–70.
- Strowski MZ, Dashkevicz MP, Parmar RM, et al., Somatostatin receptor subtypes 2 and 5 inhibit corticotropin-releasing hormone-stimulated adrenocorticotropin secretion from AtT-20 cells, *Neuroendocrinology*, 2002;75:339–46.
- Brown MR, Rivier C, Vale W, Central nervous system regulation of adrenocorticotropin secretion: role of somatostatins, *Endocrinology*, 1984;114:1546–9.
- Kraicer J, Gajewski TC, Moor BC, Release of pro-opiomelanocortin-derived peptides from the pars intermedia and pars distalis of the rat pituitary: effect of corticotrophin releasing factor and somatostatin, *Neuroendocrinology*, 1985;41:363–73.
- Voigt KH, Fehm HL, Lang RE, et al., Suppression of ACTH secretion by synthetic MSH-release inhibiting factor Pro-Leu-Gly-NH2 in Addison's disease, *Horm Metab Res*, 1977;9:150–52.
- Nicholson SA, Adrian TE, Gillham B, et al., Effect of hypothalamic neuropeptides on corticotrophin release from quarters of rat anterior pituitary gland *in vitro*, *J Endocrinol*, 1984;100:219–26.
- Lamberts SW, The role of somatostatin in the regulation of anterior pituitary hormone secretion and the use of its analogs in the treatment of human pituitary tumors, *Endocr Rev*, 1988;9:417–36.
- Lamberts SW, Uitterlinden P, Klijn JM, The effect of the long-acting somatostatin analogue SMS 201-995 on ACTH secretion in Nelson's syndrome and Cushing's disease, *Acta Endocrinol (Copenh)*, 1989;120:760–60.

18. Invitti C, de Martin M, Brunani A, et al., Treatment of Cushing's syndrome with the long-acting somatostatin analogue SMS 201-995 (sandostatin), *Clin Endocrinol (Oxf)*, 1990;32:275–81.
19. Ambrosi B, Bochicchio D, Fadin C, et al., Failure of somatostatin and octreotide to acutely affect the hypothalamic-pituitary-adrenal function in patients with corticotropin hypersecretion, *J Endocrinol Invest*, 1990;13: 257–61.
20. Stalla GK, Brockmeier SJ, Renner U, et al., Octreotide exerts different effects in vivo and in vitro in Cushing's disease, *Eur J Endocrinol*, 1994;130:125–31.
21. Schmid HA, Schoeffter P, Functional activity of the multiligand analog SOM230 at human recombinant somatostatin receptor subtypes supports its usefulness in neuroendocrine tumors, *Neuroendocrinology*, 2004;80(Suppl. 1):47–50
22. Hofland LJ, van der Hoek J, Feelders R, et al., The multi-ligand somatostatin analogue SOM230 inhibits ACTH secretion by cultured human corticotroph adenomas via somatostatin receptor type 5, *Eur J Endocrinol*, 2005;152:645–54.
23. Batista DL, Zhang X, Gejman R, et al., The effects of SOM230 on cell proliferation and adrenocorticotropin secretion in human corticotropin pituitary adenomas, *J Clin Endocrinol Metab*, 2006;91:4482–8.
24. Park S, Kamegai J, Kineman RD, Role of glucocorticoids in the regulation of pituitary somatostatin receptor subtype (sst1-sst5) mRNA levels: evidence for direct and somatostatin-mediated effects, *Neuroendocrinology*, 2003;78:163–75
25. Invitti C, Pecori Giraldi F, et al., Effect of sandostatin on CRF-stimulated secretion of ACTH, beta-lipotropin and beta-endorphin, *Horm Metab Res*, 1991;23:233–5.
26. Stafford PJ, Kopelman PG, Davidson K, et al., The pituitary-adrenal response to CRF-41 is unaltered by intravenous somatostatin in normal subjects, *Clin Endocrinol (Oxf)*, 1989;30:661–6.
27. Fehm HL, Voigt KH, Lang R, et al., Somatostatin: a potent inhibitor of ACTH-hypersecretion in adrenal insufficiency, *Klin Wochenschr*, 1976;54:173–5.
28. Julesz J, Laczi F, Janáky T, László F, Effects of somatostatin and bromocriptine on the plasma ACTH level in bilaterally adrenalectomized patients with Cushing's disease, *Endokrinologie*, 1980;76:68–72.
29. Kelestimur F, Utas C, Ozbakir O, et al., The effects of octreotide in a patient with Nelson's syndrome, *Postgrad Med J*, 1996;72:53–4.
30. Kraus J, Wöltje M, Höllt V, Regulation of mouse somatostatin receptor type 2 gene expression by glucocorticoids, *FEBS Lett*, 1999;459:200–204.
31. Ruan W, Fahlbusch F, Clemmons DR, et al., SOM230 inhibits insulin-like growth factor-I action in mammary gland development by pituitary independent mechanism: mediated through somatostatin subtype receptor 37, *Mol Endocrinol*, 2006;20:426–36.
32. Boscaro M, Ludlam WH, Atkinson B, et al., Treatment of pituitary dependent Cushing's disease with the multi-receptor ligand somatostatin analog pasireotide (SOM230): A multicenter, phase II trial, *J Clin Endocrinol Metab*, 2009;94:115–22.
33. Missale C, Nash SR, Robinson SW, et al., Dopamine receptors: from structure to function, *Physiol Rev*, 1998;78:189–225.
34. Caron MG, Beaulieu M, Raymond V, et al., Dopaminergic receptors in the anterior pituitary gland. Correlation of [3H]dihydroergocryptine binding with the dopaminergic control of prolactin release, *J Biol Chem*, 1978;253:2244–53.
35. Munemura M, Cote TE, Tsuruta K, et al., The dopamine receptor in the intermediate lobe of the rat pituitary gland: pharmacological characterization, *Endocrinology*, 1980;107: 1676–83.
36. Stefaneanu L, Kovacs K, Horvath E, et al., Dopamine D2 receptor gene expression in human adenohypophysial adenomas, *Endocrine*, 2001;14:329–36.
37. Pivonello R, Ferone D, de Herder WW, et al., Dopamine receptor expression and function in corticotroph pituitary tumors, *J Clin Endocrinol Metab*, 2004;89:2452–62.
38. Cronin MJ, Cheung CY, Wilson CB, et al., [3H]Spiperone binding to human anterior pituitaries and pituitary adenomas secreting prolactin, growth hormone, and adrenocorticotropin hormone, *J Clin Endocrinol Metab*, 1980;50:387–91.
39. Pirker W, Riedl M, Luger A, et al., Dopamine D2 receptor imaging in pituitary adenomas using iodine-123-epidepride and SPECT, *J Nucl Med*, 1996;37:1931–7.
40. Miller JW, Crapo L, The medical treatment of Cushing's syndrome, *Endocr Rev*, 1993;14:443–58.
41. Sonino N, Boscaro M, Medical therapy for Cushing's disease, *Endocrinol Metab Clin North Am*, 1999;28:211–22.
42. Adams EF, Ashby MJ, Brown SM, et al., Bromocriptine suppresses ACTH secretion from human pituitary tumour cells in culture by a dopaminergic mechanism, *Clin Endocrinol (Oxf)*, 1981;15:479–84.
43. Yin D, Kondo S, Takeuchi J, Morimura T, Induction of apoptosis in murine ACTH-secreting pituitary adenoma cells by bromocriptine, *FEBS Lett*, 1994;339:73–5.
44. Nieman LK, Medical therapy of Cushing's disease, *Pituitary*, 2002;5:77–82.
45. Bevan JS, Webster J, Burke CW, Scanlon MF, Dopamine agonists and pituitary tumor shrinkage, *Endocr Rev*, 1992;13: 204–40.
46. Kawamura M, Nakano T, Miki H, et al., Bromocriptine-responsive Cushing's disease; clinical and biochemical remission accompanied by amelioration of impaired ocular movement, *Intern Med*, 2007;46:1117–22.
47. Boscaro M, Benato M, Mantero F, Effect of bromocriptine in pituitary-dependent Cushing's syndrome, *Clin Endocrinol (Oxf)*, 1983;19:485–91.
48. Adachi M, Takayanagi R, Yanase T, et al., Cyclic Cushing's disease in long-term remission with a daily low dose of bromocriptine, *Intern Med*, 1996;35:207–11.
49. Miyoshi T, Otsuka F, Takeda M, et al., Effect of cabergoline treatment on Cushing's disease caused by aberrant adrenocorticotropin-secreting macroadenoma, *J Endocrinol Invest*, 2004;27:1055–9.
50. Ilouz F, Dubois-Ginouvès S, Laboureau S, et al., Use of cabergoline in persisting Cushing's disease, *Ann Endocrinol (Paris)*, 2006;67:353–6.
51. Pivonello R, Ferone D, de Herder WW, et al., Dopamine receptor expression and function in human normal adrenal gland and adrenal tumors, *J Clin Endocrinol Metab*, 2004;89: 4493–502.
52. Pivonello R, De Martino MC, Cappabianca P, et al., The medical treatment of Cushing's disease: effectiveness of chronic treatment with the dopamine agonist cabergoline in patients unsuccessfully treated by surgery, *J Clin Endocrinol Metab*, 2009;94:223–30.
53. Zanettini R, Antonini A, Gatto G, et al., Valvular heart disease and the use of dopamine agonists for Parkinson's disease, *N Engl J Med*, 2007;356:39–46.
54. Schade R, Andersohn F, Suissa S, et al., Dopamine agonists and the risk of cardiac-valve regurgitation, *N Engl J Med*, 2007;356: 29–38.
55. Gruszka A, Ren SG, Dong J, et al. Regulation of growth hormone and prolactin gene expression and secretion by chimeric somatostatin-dopamine molecules, *Endocrinology*, 2007;148:6107–14.
56. Krieger DT, Amorosa L, Linick F, Cyproheptadine-induced remission of Cushing's disease, *N Engl J Med*, 1975;293:893–6.
57. Suda T, Tozawa F, Mouri T, et al., Effects of cyproheptadine, reserpine, and synthetic corticotropin-releasing factor on pituitary glands from patients with Cushing's disease, *J Clin Endocrinol Metab*, 1983;56:1094–9.
58. van Waveren Hogervorst CO, Koppeschaar HP, Zelissen PM, et al., Cortisol secretory patterns in Cushing's disease and response to cyproheptadine treatment, *J Clin Endocrinol Metab*, 1996;81:652–5.
59. Tomita A, Suzuki S, Hara I, et al., Follow-up study on treatment in 27 patients with Cushing's disease: adrenalectomy, transphenoidal adenectomy and medical treatment, *Endocrinol Jpn*, 1981;28:197–205.
60. Ambrosi B, Gaggini M, Secchi F, Faglia G, Lack of effect of antiserotonergic and/or dopaminergic treatment in patients with pituitary-dependent Cushing's syndrome, *Horm Metab Res*, 1979;11:318–9.
61. Cavagnini F, Raggi U, Micossi P, et al., Effect of an antiserotonergic drug, metergoline, on the ACTH and cortisol response to insulin hypoglycemia and lysine-vasopressin in man, *J Clin Endocrinol Metab*, 1976;43:306–12.
62. Sonino N, Fava GA, Fallo F, et al., Effect of the serotonin antagonists ritanserin and ketanserin in Cushing's disease, *Pituitary*, 2000;3:55–9.
63. Tanakol R, Alagöl F, Azizlerli H, et al., Cyproheptadine treatment in Cushing's disease, *J Endocrinol Invest*, 1996;19: 242–7.
64. Whitehead HM, Beacom R, Sheridan B, Atkinson AB, The effect of cyproheptadine and/or bromocriptine on plasma ACTH levels in patients cured of Cushing's disease by bilateral adrenalectomy, *Clin Endocrinol (Oxf)*, 1990;32:193–201.
65. Koppeschaar HP, Croughs RJ, Thijssen JH, Schwarz F, Sodium valproate and cyproheptadine may independently induce a remission in the same patient with Cushing's disease, *Acta Endocrinol (Copenh)*, 1983;104:160–63.
66. Beckers A, Stevenaert A, Priens G, et al., Cyclical Cushing's disease and its successful control under sodium valproate, *J Endocrinol Invest*, 1990;13:923–9.
67. Colao A, Pivonello R, Tripodi FS, et al., Failure of long-term therapy with sodium valproate in Cushing's disease, *J Endocrinol Invest*, 1997;20:387–92.
68. Nussey SS, Price P, Jenkins JS, et al., The combined use of sodium valproate and metyrapone in the treatment of Cushing's syndrome, *Clin Endocrinol (Oxf)*, 1988;28:373–80.
69. Páez-Pereda M, Kovalovsky D, Hopfner U, et al., Retinoic acid prevents experimental Cushing syndrome, *J Clin Invest*, 2001;108:1123–31.
70. Castillo V, Giacomini D, Páez-Pereda M, et al., Retinoic acid as a novel medical therapy for Cushing's disease in dogs, *Endocrinology*, 2006;147:4438–44.
71. Philips A, Lesage S, Gingras R, et al., Novel dimeric Nur77 signaling mechanism in endocrine and lymphoid cells, *Mol Cell Biol*, 1997;17:5946–51.
72. Tran P, Zhang XK, Salbert G, et al., COUP orphan receptors are negative regulators of retinoic acid response pathways, *Mol Cell Biol*, 1992;12:4666–76.
73. Bouillier AL, Monnier D, Lorang D, et al., Corticotropin-releasing hormone stimulates proopiomelanocortin transcription by cFos-dependent and -independent pathways: characterization of an AP1 site in exon 1, *Mol Endocrinol*, 1995;9:745–55.
74. Klier SA, Umesono K, Heyman RA, et al., Retinoid X receptor-COUP-TF interactions modulate retinoic acid signaling, *Proc Natl Acad Sci U S A*, 1992;89:1448–52.
75. Cooney AJ, Leng X, Tsai SY, et al., Multiple mechanisms of chicken ovalbumin upstream promoter transcription factor-dependent repression of transactivation by the vitamin D, thyroid hormone, and retinoic acid receptors, *J Biol Chem*, 1993;268:4152–60.
76. Schwartz E, Mezick JA, Gendimenico GJ, Kligman LH, *In vivo* prevention of corticosteroid-induced skin atrophy by tretinoin in the hairless mouse is accompanied by modulation of collagen, glycosaminoglycans, and fibronectin, *J Invest Dermatol*, 1994;102:241–6.
77. Heaney AP, Fernando M, Melmed S, PPAR-gamma receptor ligands: novel therapy for pituitary adenomas, *J Clin Invest*, 2003;111:1381–8.
78. Heaney AP, Fernando M, Yong WH, Melmed S, Functional PPAR-gamma receptor is a novel therapeutic target for ACTH-secreting pituitary adenomas, *Nat Med*, 2002;8:1281–7.
79. Ambrosi B, Dall'Asta C, Cannavo S, et al., Effects of chronic administration of PPAR-gamma ligand rosiglitazone in Cushing's disease, *Eur J Endocrinol*, 2004;151:173–8.
80. Suri D, Weiss RE, Effect of pioglitazone on adrenocorticotropin hormone and cortisol secretion in Cushing's disease, *J Clin Endocrinol Metab*, 2005;90:1340–46.
81. Lemberger T, Desvergne B, Wahli W, Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology, *Annu Rev Cell Dev Biol*, 1996;12:335–63.
82. Bogazzi F, Russo D, Locci MT, et al., Peroxisome proliferator-activated receptor (PPAR)gamma is highly expressed in normal human pituitary gland, *J Endocrinol Invest*, 2005;28: 899–904.
83. Winczyk K, Pawlikowski M, Immunohistochemical detection of PPARgamma receptors in the human pituitary adenomas: correlation with PCNA, *Folia Histochem Cytobiol*, 2005;43: 137–41.
84. Emery MN, Leontiou C, Bonner SE, et al., PPAR-gamma expression in pituitary tumours and the functional activity of the glitazones: evidence that any anti-proliferative effect of the glitazones is independent of the PPAR-gamma receptor, *Clin Endocrinol (Oxf)*, 2006;65:389–95.
85. Catrina SB, Virtanen K, Hällsten K, et al., Effect of rosiglitazone on early-morning plasma cortisol levels, *Neuro Endocrinol Lett*, 2005;26:763–4.
86. Giraldi FP, Scaroni C, Arvat E, et al., Effect of protracted

- treatment with rosiglitazone, a PPAR γ agonist, in patients with Cushing's disease, *Clin Endocrinol (Oxf)*, 2006;64:219–24.
87. Bertagna X, Bertagna C, Laudat MH, et al., Pituitary-adrenal response to, to the antigluccorticoid action of RU 486 in Cushing's syndrome, *J Clin Endocrinol Metab*, 1986;63:639–43.
 88. Chu JW, Matthias DF, Belanoff J, et al., Successful long-term treatment of refractory Cushing's disease with high-dose mifepristone (RU 486), *J Clin Endocrinol Metab*, 2001;86:3568–73.
 89. Gross BA, Mindea SA, Pick AJ, et al., Medical management of Cushing disease, *Neurosurg Focus*, 2007;23:E10.
 90. Gaillard RC, Riandel A, Muller AF, et al., RU 486. A steroid with antigluccorticosteroid activity that only disinhibits the human pituitary-adrenal system at a specific time of day, *Proc Natl Acad Sci USA*, 1984;81:3879–82.
 91. Beardwell CG, Adamson AR, Shalet SM, Prolonged remission in florid Cushing's syndrome following metyrapone treatment, *Clin Endocrinol (Oxf)*, 1981;14:485–92.
 92. Boscaro M, Sonino N, Rampazzo A, Mantero F, Response of pituitary-adrenal axis to corticotrophin releasing hormone in patients with Cushing's disease before and after ketoconazole treatment, *Clin Endocrinol (Oxf)*, 1987;27:461–7.
 93. Burrin JM, Yeo TH, Ashby MJ, Bloom SR, Effect of ketoconazole on adrenocorticotrophic hormone secretion *in vitro* and *in vivo*, *J Endocrinol*, 1986;108:37–41.
 94. Gartner R, Albrecht M, Muller OA, Effect of etomidate on hypercortisolism due to ectopic ACTH production, *Lancet*, 1986;1:275.
 95. Loli P, Berselli ME, Tagliaferri M, Use of ketoconazole in the treatment of Cushing's syndrome, *J Clin Endocrinol Metab*, 1986;63:1365–71.
 96. Schteingart DE, Tsao HS, Taylor CI, et al., Sustained remission of Cushing's disease with mitotane and pituitary irradiation, *Ann Intern Med*, 1980;92:613–19.
 97. Sonino N, Boscaro M, Ambrosio G, et al., Prolonged treatment of Cushing's disease with metyrapone and aminoglutethimide, *IRCS Med Sci*, 1986;14:485–6.
 98. Sonino N, Boscaro M, Paoletta A, et al., Ketoconazole treatment in Cushing's syndrome: experience in 34 patients, *Clin Endocrinol (Oxf)*, 1991;35:347–52.
 99. Sonino N, Boscaro M, Fallo F, Pharmacologic management of Cushing syndrome: new targets for therapy, *Treat Endocrinol*, 2005;4:87–94.
 100. Sonino N, The use of ketoconazole as an inhibitor of steroid production, *N Engl J Med*, 1987;317:812–18.
 101. Verhelst JA, Trainer PJ, Howlett TA, et al., Short- and long-term responses to metyrapone in the medical management of 91 patients with Cushing's syndrome, *Clin Endocrinol*, 1991;35:169–78.
 102. Orth DN, Metyrapone is useful only as adjunctive therapy in Cushing's disease (editorial), *Ann Intern Med*, 1978;89:128–30.
 103. Misbin R, Canary J, Willard D, Aminoglutethimide in the treatment of Cushing's syndrome, *J Clin Pharmacol*, 1976;16:645–51.
 104. Zachman M, Gitzelman RP, Zagalak M, Prader A, Effect of aminoglutethimide on urinary cortisol and cortisol metabolites in adolescents with Cushing's syndrome, *Clin Endocrinol*, 1977;7:63–71.
 105. Horky K, Kuchel O, Gregorova I, Starka L, Qualitative alterations in urinary 17-ketosteroid excretion during aminoglutethimide administration, *J Clin Endocrinol Metab*, 1969;29:297–301.
 106. Ross WM, Evered DC, Hunter P, et al., Treatment of Cushing's disease with adrenal blocking drugs and megavoltage therapy to the pituitary, *Clin Radiol*, 1979;30:149–53.
 107. Thoren M, Adamson U, Sjoberg HE, Aminoglutethimide and metyrapone in the management of Cushing's syndrome, *Acta Endocrinol (Copenh)*, 1985;109:451–7.
 108. Jeffcoate WJ, Rees LH, Tomlin S, et al., Metyrapone in long-term management of Cushing's disease, *Br J Med*, 1977;2:215–17.
 109. Dickstein G, Lahav M, Shen-Orr Z, Edoute Y, Barzilai D, Primary therapy for Cushing's disease with metyrapone, *JAMA*, 1986;255:1167–9.
 110. Donckier J, Burrin JM, Ramsay ID, Joplin GF, Successful control of Cushing's disease in the elderly with long term metyrapone, *Postgrad Med J*, 1986;62:727–30.
 111. Child DF, Burke CW, Burley DM, et al., Drug control of Cushing's syndrome: Combined aminoglutethimide and metyrapone therapy, *Acta Endocrinol (Copenh)*, 1976;82:330–41.
 112. Dang CN, Trainer P, Pharmacological management of Cushing's syndrome: an update, *Arq Bras Endocrinol Metabol*, 2007;51:1339–48.
 113. Morris D, Grossman A, The medical management of Cushing's syndrome, *Ann N Y Acad Sci*, 2002;970:119–33.
 114. Trainer PJ, Eastment C, Grossman AB, et al., The relationship between cortisol production rate and serial serum cortisol estimation in patients on medical therapy for Cushing's syndrome, *Clin Endocrinol (Oxf)*, 1993;39:441–3.
 115. Connell JM, Cordiner J, Davies DL, et al., Pregnancy complicated by Cushing's syndrome: potential hazard of metyrapone therapy. Case report, *Br J Obstet Gynaecol*, 1985;92:1192–5.
 116. Feldman D, Ketoconazole and other imidazole derivatives as inhibitors of steroidogenesis, *Endocr Rev*, 1986;7:409–20.
 117. Loose DS, Kan PB, Hirst MA, et al., Ketoconazole blocks adrenal steroidogenesis by inhibiting cytochrome P450-dependent enzymes, *J Clin Invest*, 1983;71:1495–9.
 118. Jimenez Reina L, Leal-Cerro A, Garcia J, et al., *In vitro* effects of ketoconazole on corticotrope cell morphology and ACTH secretion of two pituitary adenomas removed from patients with Nelson's syndrome, *Acta Endocrinol (Copenh)*, 1989;121:185–90.
 119. Stalla GK, Stalla J, Huber M, et al., Ketoconazole inhibits corticotrophic cell function *in vitro*, *Endocrinology*, 1988;122:618–23.
 120. Loose DS, Stover EP, Feldman D, Ketoconazole binds to glucocorticoid receptors and exhibits glucocorticoid antagonist activity in cultured cells, *J Clin Invest*, 1983;72:404–8.
 121. Pardes E, De Yampye JE, Moses DF, De Nicola AF. Regulation of glucocorticoid receptors in human mononuclear cells: effects of glucocorticoid treatment, Cushing's disease and ketoconazole, *J Steroid Biochem Mol Biol*, 1991;39:233–8.
 122. Engelhardt D, Weber MM, Therapy of Cushing's syndrome with steroid biosynthesis inhibitors, *J Steroid Biochem Mol Biol*, 1994;49:261–7.
 123. Vignati F, Loli P, Additive effect of ketoconazole and octreotide in the treatment of severe adrenocorticotropin-dependent hypercortisolism, *J Clin Endocrinol Metab*, 1996;81:2885–90.
 124. Angeli A, Frairia R, Ketoconazole therapy in Cushing's disease, *Lancet*, 1985;1:821.
 125. Duarte PA, Chow CC, Simmons F, Ruskin J, Fatal hepatitis associated with ketoconazole therapy, *Arch Intern Med*, 1984;144:1069–70.
 126. Knight TE, Shikuma CY, Knight J, Ketoconazole-induced fulminant hepatitis necessitating liver transplantation, *J Am Acad Dermatol*, 1991;25:398–400.
 127. Lewis JH, Hyman JZ, Benson GD, Ishak KG, Hepatic injury associated with ketoconazole therapy, *Gastroenterology*, 1984;86:503–13.
 128. Stricker BH, Blok AP, Bronkhorst FB, et al., Ketoconazole-associated hepatic injury. A clinicopathological study of 55 cases, *J Hepatol*, 1986;3:399–406.
 129. McCance DR, Ritchie CM, Sheridan B, Atkinson AB, Acute hypoadrenalism and hepatotoxicity after treatment with ketoconazole, *Lancet*, 1987;1:573.
 130. Castinetti F, Morange I, Jaquet P, et al., Ketoconazole revisited: a preoperative or postoperative treatment in Cushing's disease, *Eur J Endocrinol*, 2008;158:91–9.
 131. Santen RJ, Misbin RI, Aminoglutethimide: Review of pharmacology and clinical use, *Pharmacotherapy*, 1981;1:95–120.
 132. Cash R, Brough AJ, Cohen MN, Satoh PS, Aminoglutethimide (Elipten-Ciba) as an inhibitor of adrenal steroidogenesis: mechanism of action and therapeutic trial, *J Clin Endocrinol Metab*, 1967;27:1239–48.
 133. Dexter RN, Fishman LM, Ney RL, Liddle GW, Inhibition of adrenal corticosteroid synthesis by aminoglutethimide: studies of the mechanism of action, *J Clin Endocrinol Metab*, 1967;27:473–80.
 134. Shaw MA, Nicholls PJ, Smith HJ, Aminoglutethimide and ketoconazole: historical perspectives and future prospects, *J Steroid Biochem*, 1988;31:137–46.
 135. Schteingart DE, Conn JW, Effects of aminoglutethimide upon adrenal function and cortisol metabolism in Cushing's syndrome, *J Clin Endocrinol Metab*, 1967;27:1657–66.
 136. Santen RJ, Wells SA, Runic S, et al., Adrenal suppression with aminoglutethimide. I. Differential effects of aminoglutethimide on glucocorticoid metabolism as a rationale for use of hydrocortisone, *J Clin Endocrinol Metab*, 1977;45:469–79.
 137. Weber MM, Lang J, Abedinpour F, et al., Different inhibitory effect of etomidate and ketoconazole on the human adrenal steroid biosynthesis, *Clin Invest*, 1993;71:933–8.
 138. Lamberts SW, Bons EG, Bruining HA, de Jong FH, Differential effects of the imidazole derivatives etomidate, ketoconazole and miconazole and of metyrapone on the secretion of cortisol and its precursors by human adrenocortical cells, *J Pharmacol Exp Ther*, 1987;240:259–64.
 139. De Coster R, Beerens D, Haelterman C, Wouters L, Effects of etomidate on cortisol biosynthesis in isolated guinea-pig adrenal cells: comparison with metyrapone, *J Endocrinol Invest*, 1985;8:199–202.
 140. Alolio B, Stuttmann R, Fischer H, et al., Long-term etomidate, and adrenocortical suppression, *Lancet*, 1983;2:626.
 141. Klauson NO, Moelgaard J, Ferguson AH, et al., Negative synacthen test during etomidate infusion, *Lancet*, 1983;2:848.
 142. Lambert A, Mitchell R, Frost J, et al., Direct *in vitro* inhibition of adrenal steroidogenesis by etomidate, *Lancet*, 1983;2:1085–6.
 143. Fraser R, Watt I, Gray CE, et al., The effect of etomidate on adrenocortical function in dogs before and during hemorrhagic shock, *Endocrinology*, 1984;115:2266–70.
 144. Drake WM, Perry LA, Hinds CJ, et al., Emergency and prolonged use of intravenous etomidate to control hypercortisolemia in a patient with Cushing's syndrome and peritonitis, *J Clin Endocrinol Metab*, 1998;83:3542–4.
 145. Schulte HM, Benker G, Reinwein D, et al., Infusion of low dose etomidate: Correction of hypercortisolemia in patients with Cushing's syndrome and dose-response relationship in normal subjects, *J Clin Endocrinol Metab*, 1990;70:1426–30.
 146. Krakoff J, Koch CA, Calis KA, et al., Use of a parenteral propylene glycol-containing etomidate preparation for the long-term management of ectopic Cushing's syndrome, *J Clin Endocrinol Metab*, 2001;86:4104–8.
 147. Johnson TN, Canada TW, Etomidate use for Cushing's syndrome caused by an ectopic adrenocorticotrophic hormone-producing tumor, *Ann Pharmacother*, 2007;41:350–53.
 148. Greening JE, Brain CE, Perry LA, et al., Efficient short-term control of hypercortisolemia by low-dose etomidate in severe paediatric Cushing's disease, *Horm Res*, 2005;64:140–43.
 149. Potts GO, Creange JE, Harding HR, Schane HP, Trilostane, an orally active inhibitor of steroid biosynthesis, *Steroids*, 1978;32:257–67.
 150. Neiger R, Ramsey I, O'Connor J, et al., Trilostane treatment of 78 dogs with pituitary-dependent hyperadrenocorticism, *Vet Rec*, 2002;150:799–804.
 151. Dewis P, Anderson DC, Bu'lock DE, et al., Experience with trilostane in the treatment of Cushing's syndrome, *Clin Endocrinol (Oxf)*, 1983;18:533–40.
 152. Hart MM, Swackhamer ES, Straw JA, Studies on the site of action of, p_-DDD in the dog adrenal cortex: TNPHand corticosteroid precursor stimulation of o, p'-DDD inhibited steroidogenesis, *Steroids*, 1971;17:575–86.
 153. Ojima M, Saitoh M, Itoh N, et al., The effects of o, p'-DDD on adrenal steroidogenesis and hepatic steroid metabolism, *Nippon Naibunpi Gakkai Zasshi Folia Endocrinol Jpn*, 1985;61:168–78.
 154. Hart MM, Straw JA, Studies on the site of action of o, p'-DDD in the dog adrenal cortex. 1. Inhibition of ACTH-mediated pregnenolone synthesis, *Steroids*, 1971;17:559–74.
 155. Luton JP, Mahoudeau JA, Bouchard P, et al., Treatment of Cushing's disease by o, p'-DDD: Survey of 62 cases, *N Engl J Med*, 1979;300:459–64.
 156. Trainer PJ, Besser M, Cushing's syndrome. Therapy directed at the adrenal glands, *Endocrinol Metab Clin North Am*, 1994;23:571–84.
 157. Hague RV, May W, Cullen DR, Hepatic microsomal enzyme induction and adrenal crisis due to o, p'-DDD therapy for metastatic adrenocortical carcinoma, *Clin Endocrinol*, 1989;31:51–7.
 158. Leiba S, Weinstein R, Shindel B, et al., The protracted effect of o, p'-DDD in Cushing's disease and its impact on adrenal morphogenesis of young human embryo, *Ann Endocrinol*, 1989;50:49–53.
 159. Hutter AM, Kayhoe DE, Adrenal cortical carcinoma: Results of treatment with o, p_-DDD in 138 patients, *Am J Med*, 1966;41:581–92.
 160. Robinson BG, Hales IB, Henniker AJ, et al., The effect of o, p'-DDD on adrenal steroid replacement therapy requirements, *Clin Endocrinol (Oxf)*, 1987;27:437–44.