0021-972X/07/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 92(1):65–69

doi: 10.1210/jc.2006-0728

Dopamine Receptor Expression and Function in Corticotroph Ectopic Tumors

Rosario Pivonello, Diego Ferone, Wouter W. de Herder, Antongiulio Faggiano, Lisa Bodei, Ronald R. de Krijger, Gaetano Lombardi, Annamaria Colao, Steven W. J. Lamberts, and Leo J. Hofland

Departments of Internal Medicine (R.P., D.F., W.W.d.H., S.W.J.L., L.J.H.) and Pathology (R.R.d.K.), Erasmus Medical Center, 3015 GE Rotterdam, The Netherlands; Departments of Molecular and Clinical Endocrinology and Oncology (R.P., A.F., G.L., A.C.), "Federico II" University of Naples, 80131 Naples, Italy; Department of Nuclear Medicine, European Institute of Oncology (L.B.), 20141 Milan, Italy; and Department of Endocrinological and Metabolic Sciences and Center of Excellence for Biomedical Research (D.F.), University of Genoa, 16132 Genoa, Italy

Background: Dopamine receptor (DR) expression and dopamine agonist (DA) effectiveness have never been demonstrated in neuroendocrine tumors associated with ectopic ACTH syndrome (EAS).

Aim: The aim of the current study was to evaluate DR and particularly D_2 subtype expression in neuroendocrine tumors associated with EAS and to evaluate the *in vivo* effectiveness of the DA cabergoline in the treatment of EAS.

Patients and Methods: Six ACTH-secreting neuroendocrine tumors, including four lung, one pancreatic, and one thymic carcinoid, were used for the evaluation of D_2 expression by immunohistochemistry. DR subtypes and D_2 isoforms and number were evaluated by RT-PCR in three cases of persistent EAS after surgery. These patients were treated with cabergoline at the dose of 3.5 mg/wk for 6 months. Clinical parameters, hormonal levels, and tumor size were monitored during the treatment period.

Results: At immunohistochemistry, D_2 was expressed in five (83.3%) tumors. At RT-PCR, D_2 was confirmed in all three cases but at variable numbers, whereas D_4 was expressed in two cases. D_{2long} was expressed in all three cases, together with D_{2short} in one case. A normalization of urinary cortisol levels was found in two patients (66.7%) after 3 months of treatment. However, treatment escape was demonstrated in one of these patients afterward.

Conclusion: The results of this study demonstrated that DR are expressed in neuroendocrine tumors associated with EAS and that cabergoline treatment could be effective in controlling cortisol excess in a subgroup of patients with EAS. Further studies on a larger number of patients are mandatory to confirm the usefulness of DA in EAS. **(*J Clin Endocrinol Metab* 92: 65–69, 2007)**

THE ECTOPIC ACTH syndrome (EAS) is a rare cause of Cushing syndrome (CS). Neuroendocrine tumors, localized in lung, thymus, or gastro-entero-pancreatic system, frequently classified as carcinoids, represent the main causes of EAS (1). The treatment of choice of EAS is the surgical removal of the tumor, but its success rate is limited because of persistent tumor remnants, especially in case of lung carcinoids (1, 2). Medical treatment is generally palliative and aimed at inhibiting adrenal cortisol secretion (3). Moreover, somatostatin analogs (SA) were found to be effective in controlling the carcinoid ACTH secretion after short-term treatment but to fail frequently in maintaining this control after long-term treatment (4). Dopamine receptors (DR) have been postulated in neuroendocrine tumors (5) and demonstrated to be expressed in ACTH-secreting pituitary tumors (6). Dopamine agonists (DA), although experimentally used in the treatment of pituitary-dependent CS (6), have been never used in the treatment of EAS. The aim of the current study was to evaluate DR expression in ACTH-secreting extrapituitary (ectopic) neuroendocrine tumors associated with EAS and the effectiveness of the DA cabergoline on the control of ACTH and cortisol secretion in three cases unsuccessfully operated and with persistent EAS after surgery.

First Published Online October 10, 2006

Abbreviations: CS, Cushing syndrome; DA, dopamine agonist(s); DR, dopamine receptor(s); EAS, ectopic ACTH syndrome; HPRT, hypoxanthine phosphoribosyltransferase; SA, somatostatin analog(s).

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

Patients and Methods

Patients

Six patients (two females and four males, 30–50 yr old) with a diagnosis of EAS associated with ACTH-secreting neuroendocrine tumors entered the study after their informed consent had been obtained. All patients were subjected to surgery for the removal of the tumor. The histological and immunohistochemical study of the tumor documented an ACTH-secreting neuroendocrine tumor in all cases: a lung carcinoid in four cases (typical in three and atypical in one) and a pancreatic well-differentiated endocrine carcinoma (pancreatic carcinoid) and a thymic carcinoid in the remaining two cases. The patients' profile and tumor characteristics are shown in Table 1. After surgery, a clinical, hormonal, and radiological remission of EAS was documented in three patients, whereas disease persisted in the remaining three patients (patients 1–3 in Table 1), all bearing a residual lung carcinoid (typical in two and atypical in one). In these three patients, at the diagnosis, tumor diameters were 1.8 cm (patient 1), 1.5 cm (patient 2), and 1.2 cm (patient 3). Lymph node metastases were present in two patients (patients 1 and 2), whereas no distant metastases were detected in any of the three patients. After surgery, a residual tumor was found in all three cases: tumor diameter was measurable (1.0 cm) only in one case (patient 1), where positive lymph nodes were also found, whereas in the remaining two patients residual tumors were not visible with standard imaging techniques but were clearly visualized with somatostatin receptor scintigraphy.

TABLE 1. Histological diagnosis, IHC, and RT-PCR results in the ACTH-secreting tumors deriving from patients with EAS

Cases	Histology	IHC			RT-PCR						
		Chromogranin	ACTH	D_2	D_1	D_2	D_2 isoforms	D_2 number[a]	D_3	D_4	D_5
1	Lung carcinoid	+++	+++	+++	−	+	S/L	0.16	−	+	−
2	Lung carcinoid	++	+	++	−	+	L	0.08	−	+	−
3	Lung carcinoid	++	++	+	−	+	L	0.07	−	−	−
4	Lung carcinoid	++	+	+++	nd	nd	nd	nd	nd	nd	nd
5	Thymic carcinoid	++	++	+	nd	nd	nd	nd	nd	nd	nd
6	Pancreatic carcinoid	++	+	−	nd	nd	nd	nd	nd	nd	nd

IHC: +, weakly positive; ++, moderately positive; +++, strongly positive; −, negative; RT-PCR: +, positive; −, negative; S, short isoform; L, long isoform; nd, not determined.

[a] The D_2 receptor has been explained as the ratio between the number of copy of messenger RNA of D_2 and the number of copy of mRNA of HPRT. The D_2 number in five cases of ACTH-secreting pituitary tumors, used as controls, was 1.57 ± 1.10 (range, 0.09–6.0).

Study design

DR subtype and D_2 isoform (D_{2long} and D_{2short}) expression were evaluated by standard RT-PCR and D_2 number was evaluated by quantitative RT-PCR in three cases (patients 1–3 in Table 1), whereas D_2 receptor expression and localization was evaluated by immunohistochemistry (IHC) in all six cases. The effectiveness of cabergoline treatment on clinical, hormonal, and radiological parameters was evaluated in the three cases of patients with persistent EAS after surgery (patients 1–3 in Table 1). The study was in accordance with the Helsinki Doctrine on Human Experimentation and the protocol was approved by the Local Ethical Committees.

IHC

Tumor specimens, obtained at the time of tumor excision, were fixed in formalin and embedded in paraffin for IHC study. The IHC study was performed on tissue samples according to previous reports (6, 7). Immunostaining for D_2 was performed using a rabbit antihuman polyclonal antibody (Chemicon International, Temecula, CA) in a dilution of 1:500. Immunostaining for ACTH (Neomarkers, Duiven, The Netherlands; dilution 1:100), chromogranin A (Biogenix, Duiven, The Netherlands; dilution 1:100), and synaptophysin (Dako, Heverlee, Belgium; dilution 1:50) was performed, together with histology, on sequential sections. The intensity of immunostaining for ACTH, chromogranin A, synaptophysin, and D_2 receptor was scored with the following semiquantitative method: −, absent; +, weak; ++, moderate; +++, strong immunostaining.

RT-PCR

Tumor specimens, obtained at the time of tumor excision, were quickly frozen on dry ice and stored in a freezer at −80 C for RT-PCR study. Standard RT-PCR was performed according to previous reports (6, 7). Quantitative RT-PCR was performed according to a previous report (8), after the isolation of total RNA, performed by using the Tri Pure Isolation Reagent kit (Roche Molecular Biochemicals, Indianapolis, IN). The detection of hypoxanthine phosphoribosyltransferase (HPRT) served as a control and was used for normalization of the D_2 levels. Primer and probe sequence for HPRT has been previously described (9). Primers and probe for D_2 were: 5′-GCCACTCAGATGCTCGCC-3′ (forward), 5′-ATGTGTGTGATGAAGAAGGGCA-3′ (reverse) and 5′-FAM-TTGTTCTCGGCGTGTTCATCATCTGC-TAMRA-3′ (probe). Primers and probe for HPRT were: 5′-TGCTTTCCTTGGTCAGGCAGTAT-3′ (forward), 5′-TCAAATCCAACAAAGTCTGGCTTATATC-3′ (reverse), and 5′-FAM-CAAGCTTGCGACCTTGACCATCTTTGGA-TAMRA-3′ (probe). Five cases of ACTH-secreting pituitary tumors were used as controls for the evaluation of the D_2 number at the quantitative RT-PCR.

Therapeutic study

Cabergoline was administered at the dose of 3.5 mg/wk (0.5 mg/d), after the administration of a test dose (1 mg) during the week before starting the treatment to evaluate the tolerability for the drug. The clinical evaluation, including the scoring of the symptoms and signs, and mainly the measurement of blood pressure and serum glucose and lipid levels as well as the hormonal evaluation, including the measurement of plasma ACTH and serum and urinary cortisol levels, were performed monthly, whereas the radiological evaluation, including the measurement of tumor mass, was performed every 3 months and/or at the withdrawal of the cabergoline treatment by computed tomography or magnetic resonance imaging.

Hormone assay

Plasma ACTH levels were measured by immunoradiometric assay, whereas serum and urinary cortisol levels were measured by RIA method using commercially available kits. Both at baseline and during the follow-up evaluations, plasma ACTH and serum cortisol were measured after an overnight fasting at 0800 h in a single sample, whereas urinary cortisol levels were collected three times in the same week and the mean considered for the study. Normal ranges were 10–100 pg/ml for plasma ACTH, 50–200 μg/liter for serum cortisol and 35–135 μg/24 h for urinary cortisol.

Results

DR expression

In the IHC study, specific D_2 immunostaining was found in five (83.3%) of the six cases, including the four lung and the thymic carcinoids. D_2 immunostaining was strong and homogeneous in two lung carcinoids, moderate in one lung carcinoid, and weak and heterogeneous in one lung and in the thymic carcinoid. In the standard RT-PCR study, D_2 receptor was expressed in all three cases (lung carcinoids) that were tested with this technique (both D_{2long} and D_{2short} in one case and only D_{2long} in two cases), whereas D_4 receptor was expressed in two of the three lung carcinoids (patients 1 and 2 in Table 1). In the quantitative RT-PCR study, a variable number of D_2 was found, being relatively higher in one case (patient 1 in Table 1) and lower in two cases (patients 2 and 3 in Table 1). The results of the RT-PCR and IHC studies are summarized in Table 1. An example of the IHC results in one case of lung carcinoid (patient 4 in Table 1) is shown in Fig. 1.

DA effectiveness

A significant inhibition of plasma ACTH, as well as serum and urinary cortisol levels, was found in two of the three (66.7%) patients with postoperative persistent EAS after 1 month, and a complete normalization of both ACTH and cortisol levels was found in these patients after 3 months of treatment with cabergoline. Conversely, no significant hormonal changes were found in the third patient, in whom, therefore, treatment was stopped after 1 month. In one of the two responsive patients, ACTH started to rise again at 4

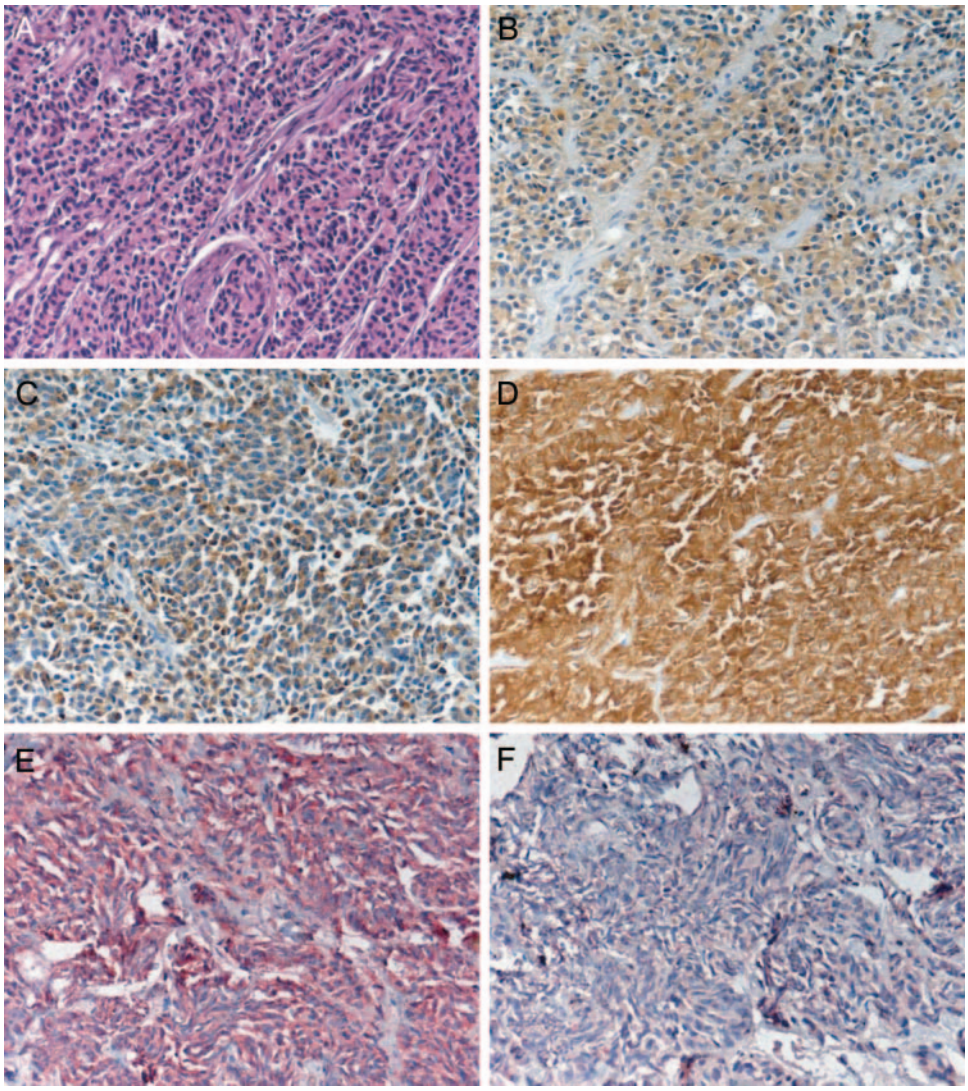

FIG. 1. Example of the result of IHC study in a case of ACTH-secreting lung carcinoid (patient 4 in Table 1). The picture includes histology with hematoxylin eosin staining (A), ACTH (B), chromogranin (C), and D_2 receptor (D) immunostaining. Displacement of immunostaining after preabsorption of the D_2 antibody with immunizing peptide, demonstrating specificity of the staining, is shown in photomicrograph E. The picture shows a diffuse immunostaining for chromogranin and ACTH within the lung tumor sample, confirming the diagnosis of ACTH-secreting carcinoid tumor. The carcinoid displays a significant immunostaining for D_2 receptor.

months, whereas cortisol started to reincrease at 5 months of treatment and did not normalize despite the augmentation of the cabergoline dose from 0.5 to 1 mg/d. In this patient cabergoline treatment was stopped after 6 months of treatment. In the remaining responsive patient, ACTH and cortisol levels, despite some fluctuations, remained normal during the 6 months of treatment. Plasma ACTH and urinary cortisol levels in the three patients treated with cabergoline are shown in Fig. 2. A significant improvement of the clinical syndrome, mainly blood pressure and blood glucose levels, was found in the two responsive but not in the resistant patient. In the patient who had a treatment escape, a reworsening of the clinical syndrome was found at the 6-month follow-up. No change in tumor size was found in any of the three patients during the treatment period.

Discussion

EAS is a rare but severe syndrome frequently caused by neuroendocrine tumors, recently defined as endocrine tumors or carcinomas, generally localized in the chest or derived from the gastro-entero-pancreatic system (1). The most common tumor causing EAS is the lung carcinoid (1). The optimal treatment of EAS associated with lung carcinoid is surgical removal of the tumor and related resectable metastases (1). However, surgery does not represent a curative treatment in the majority of patients. Alternative treatments are generally palliative. Because chemotherapy is not effective except in aggressive tumors, treatments targeting the adrenal glands and aiming at normalizing cortisol secretion are the most commonly used therapies when the source of ACTH secretion cannot be found, removed, or treated (3). SA have recently taken a central place in the treatment of EAS caused by lung carcinoids, acting through somatostatin receptors frequently expressed in these tumors (4, 10). However, although short-term efficacy of octreotide in controlling ACTH and cortisol secretion has been described in many cases of EAS, reports of long-term effectiveness of octreotide are limited, probably because of the frequent occurrence of treatment escape (11–13).

Despite the well-known DR expression and role in pituitary tumors (6, 14–18), the DR expression and its role in extrapituitary (ectopic) neuroendocrine tumors, and partic-

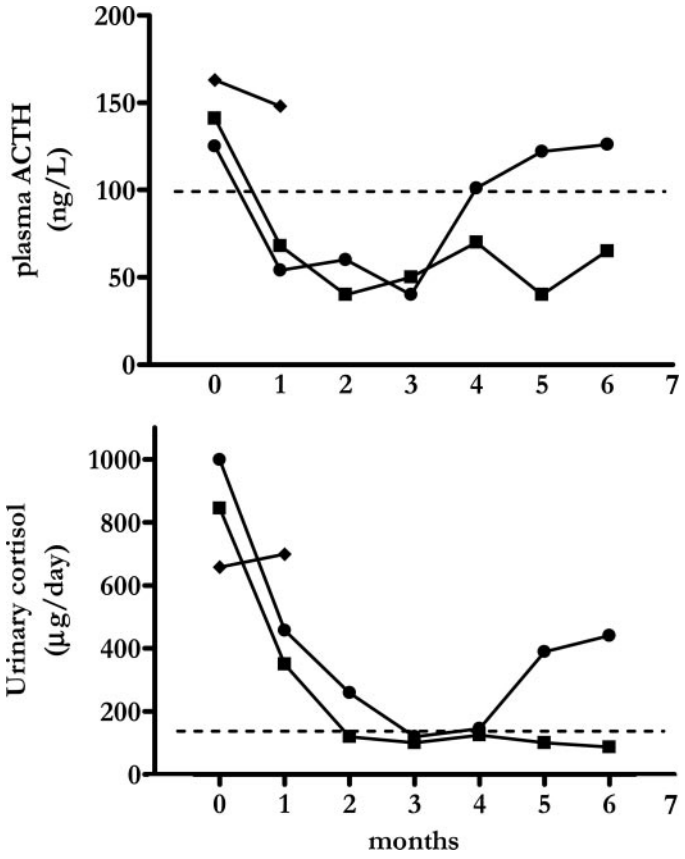

FIG. 2. Plasma ACTH (*top*) and urinary cortisol (*bottom*) response to cabergoline treatment in three patients with persistent EAS due to an ACTH-secreting lung carcinoid (■, patient 1; ●, patient 2; ▲, patient 3 in Table 1). The *interrupted line* indicates the upper limit of the normal range.

ularly in carcinoids, has never been extensively evaluated. Furthermore, despite the demonstrated DA effectiveness in the treatment of pituitary-dependent CS (6, 19), the possible DA effectiveness in the treatment of EAS has never been tested. DR represent a group of five different G protein-coupled receptors (D_1–D_5), mediating the various central and peripheral actions of dopamine (20). DR expression and dopamine synthesis have been demonstrated in gastrointestinal neuroendocrine tumor cell lines (5). The current study is the first demonstrating DR, particularly D_2 and D_4, expression in ACTH-secreting ectopic tumors, including lung, thymus, and pancreatic carcinoids, suggesting that DR may play a role in the biology of these tumors and/or form a target for treatment with DA.

Cabergoline was demonstrated to be effective in the treatment of ACTH-secreting pituitary tumors, causing CS (6). The current study demonstrates for the first time a possible effectiveness of cabergoline in the treatment of ACTH-secreting ectopic tumors, causing EAS. Short-term cabergoline treatment significantly inhibited ACTH, and consequently cortisol secretion in two of three cases with EAS due to a lung carcinoid. Hormonal suppression was maintained for a long-term follow-up in one of these two cases, with a clear-cut improvement of clinical syndrome and without any significant change in the size of the tumor. It is noteworthy that the patient not responsive to cabergoline was bearing a tumor expressing only the long isoform of D_2 and the patient who experienced a treatment escape was bearing a tumor expressing the long isoform of D_2 associated with D_4 receptor, whereas the patient showing long-term responsiveness to cabergoline, was bearing a tumor expressing both isoforms of D_2 as well as D_4 receptors. Taken into consideration that three cases are not enough to draw final conclusions, it seems that the expression of short isoform of D_2 and/or the coexpression of D_4 may play a pivotal role in the effectiveness of DA in the treatment of carcinoid tumors associated with EAS. This hypothesis seems to be supported by similar evidence found in the treatment of pituitary tumors (6, 17). Alternatively, the responsiveness and/or the protection from treatment escape could be related to the different numbers of D_2 and/or D_4 expressed in the tumor. Indeed, it has to be pointed out that the patient who was long-term responsive to cabergoline treatment had a tumor with a stronger D_2 at IHC and a relatively higher number of D_2 at RT-PCR than the nonlong-term responsive patients.

Finally, the possible effectiveness of DA, demonstrated in the present study, and the well-described effectiveness of SA, demonstrated in previous studies, together with the demonstration of a synergistic cooperation between somatostatin receptors and DR and the potentiation of the correspondent SA and DA effectiveness in transfected cell lines (21), suggest a possible synergism in the action of the two categories of drugs in the treatment of carcinoid tumors causing EAS. This phenomenon has been indeed recently demonstrated in a case of EAS due to lung carcinoid (22).

In conclusion, DR, particularly D_2 and D_4, are expressed in carcinoid tumors associated with EAS, where they can mediate a therapeutic effect of DA, particularly cabergoline, in the inhibition of ACTH and cortisol secretion, and so preventing the complications of glucocorticoids excess due to EAS. These data suggest that DA might be included in the therapeutic options, especially in combination with SA, of persisting EAS, mainly for patients with occult or nonresectable tumors or patients with active disease before surgery or disease persistence after surgery, who are waiting for the definitive cure. It is important to point out that further studies on a larger number of patients are mandatory to confirm the real usefulness of DA in the management of EAS.

Acknowledgments

We are greatly indebted to Pharmacia-Pfizer for its technical support of the study.

Received April 4, 2006. Accepted September 29, 2006.

Address all correspondence and requests for reprints to: Rosario Pivonello, M.D., Ph.D., Department of Molecular and Clinical Endocrinology and Oncology, "Federico II" University, Via Sergio Pansini, 5, 80131 Naples, Italy. E-mail: rpivone@tin.it.

The work was supported by Pharmacia-Pfizer and in part by a grant from the Italian Minister of University and Research (2001-RBAU01FMEY).

Disclosure Statement: The authors have nothing to disclose.

References

1. **Wajchenberg BL, Mendonca BB, Liberman B, Pereira MA, Carneiro PC, Wakamatsu A, Kirschner MA** 1994 Ectopic adrenocorticotropic hormone syndrome. Endocr Rev 15:752–787

2. **Pass HI, Doppman JL, Nieman LK, Stovroff M, Vetto J, Norton JA, Travis W, Chrousos GP, Oldfield EH, Cutler GB** 1990 Management of the ectopic ACTH syndrome due to thoracic carcinoids. Ann Thor Surg 50:52–57
3. **Miller JW, Crapo L** 1993 The medical treatment of Cushing's syndrome. Endocr Rev 14:443–458
4. **von Werder K, Muller OA, Stalla GK** 1996 Somatostatin analogs in ectopic corticotropin production. Metabolism 45:129–131
5. **Lemmer K, Ahnert-Hilger G, Hopfner M, Hoegerle S, Faiss S, Grabowski P, Jockers-Scherubl M, Riecken EO, Zeitz M, Scherubl H** 2002 Expression of dopamine receptors and transporter in neuroendocrine gastrointestinal tumor cells. Life Sci 71:667–678
6. **Pivonello R, Ferone D, de Herder WW, Kros JM, Del Basso De Caro ML, Arvigo M, Annunziato L, Lombardi G, Colao A, Hofland LJ, Lamberts SWJ** 2004 Dopamine receptor expression and function in corticotroph pituitary tumors. J Clin Endocrinol Metab 89:2452–2462
7. **Pivonello R, Ferone D, de Herder WW, de Krijger RR, Waaijers M, Mooij DM, van Koetsveld PM, Barreca A, Del Basso De Caro ML, Lombardi G, Colao A, Lamberts SWJ, Hofland LJ** 2004 Dopamine receptor expression and function in human normal adrenal gland and adrenal tumors. J Clin Endocrinol Metab 89:4493–4502
8. **Ferone D, Pivonello R, Van Hagen PM, Dalm VA, Lichtenauer-Kaligis EG, Waaijers M, Van Koetsveld PM, Mooy DM, Colao A, Minuto F, Lamberts SW and Hofland LJ** 2002 Quantitative and functional expression of somatostatin receptor subtypes in human thymocytes. Am J Physiol Endocrinol Metab 283:E1056–E1066
9. **Hofland LJ, van der Hoek J, van Koetsveld PM, de Herder WW, Waaijers M, Sprij-Mooij D, Bruns C, Weckbecker G, Feelders R, van der Lely AJ, Beckers A, Lamberts SW** 2004 The novel somatostatin analog SOM230 is a potent inhibitor of hormone release by growth hormone- and prolactin-secreting pituitary adenomas in vitro. J Clin Endocrinol Metab 89:1577–1585
10. **Hofland LJ, Lamberts SWJ** 2001 Somatostatin receptor subtype expression in human tumors. Ann Oncol 12(Suppl 2): S31–S36
11. **Van den Bruel A, Bex M, Van Dorpe J, Heyns W, Bouillon R** 1998 Occult ectopic ACTH secretion due to recurrent lung carcinoid: long-term control of hypercortisolism by continuous subcutaneous infusion of octreotide. Clin Endocrinol (Oxf) 49:541–546
12. **de Herder WW, Lamberts SWJ** 2002 Somatostatin and somatostatin analogues: diagnostic and therapeutic uses. Curr Opin Oncol 14:53–57
13. **Hofland LJ, Lamberts SWJ** 2003 The pathophysiological consequences of somatostatin receptor internalisation and resistance. Endocr Rev 24:28–47
14. **Cronin MJ, Evans WS** 1983 Dopamine receptors in the normal and abnormal anterior pituitary gland. Clin Endocrinol Metab 12:15–30
15. **Colao A, Ferone D, Marzullo P, Di Sarno A, Cerbone G, Sarnacchiaro F, Cirillo S, Merola B, Lombardi G** 1997 Effect of different dopaminergic agents in the treatment of acromegaly. J Clin Endocrinol Metab 82:518–523
16. **Colao A, Di Sarno A, Pivonello R, Di Somma C, Lombardi G** 2002 Dopamine receptor agonist for treating prolactinomas. Exp Opin Invest Drugs 11:787–800
17. **Pivonello R, Matrone C, Filippella M, Cavallo LM, Di Somma C, Cappabianca P, Colao A, Annunziato L, Lombardi G** 2004 Dopamine receptor expression and function in clinically nonfunctioning pituitary tumors: comparison with the effectiveness of cabergoline treatment. J Clin Endocrinol Metab 89:1674–1683
18. **Bevan JS, Webster J, Burke CW, Scanlon MF** 1992 Dopamine agonists and pituitary tumor shrinkage. Endocr Rev 13:220–240
19. **Lamberts SWJ, Klijn JG, de Quijada M, Timmermans HA, Uitterlinden P, de Jong FH, Birkenhager JC** 1980 The mechanism of the suppressive action of bromocriptine on adrenocorticotropin secretion in patients with Cushing's disease and Nelson's syndrome. J Clin Endocrinol Metab 51:307–311
20. **Missale C, Nash SR, Robinson SW, Jaber M, Caron MG** 1998 Dopamine receptors: from structure to function. Physiol Rev 78:189–225
21. **Rocheville M, Lange DC, Kumar U, Patel SC, Patel RC, Patel YC** 2000 Receptor for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. Science 288:154–157
22. **Pivonello R, Ferone D, Lamberts SWJ, Colao A** 2005 Cabergoline *plus* lanreotide for ectopic Cushing's syndrome. N Engl J Med 352:2457–2458

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.