Role of Dopamine Receptors in Normal and Tumoral Pituitary Corticotrophic Cells and Adrenal Cells

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Abstract
The recent depiction of dopamine receptors (DRs) in tumors that cause Cushing’s syndrome (CS) has renewed the debate about the dopamine control on pituitary-adrenal axis, and opened interesting new perspectives for medical treatment of CS. The new insights arise from the recent accurate characterization of DR subtypes expression within tumors causing CS, the discovery of new mechanisms, such as the dimerization between DRs and other G-protein coupled receptors (CPCRs), including somatostatin receptors (SSTRs), and the recent availability of new agents targeting these receptor subtypes. Corticotrophic adenomas express DR subtype 2 (D2R), together with different SSTR subtypes (ssts), in particular sst5. In vitro, activation of D2R inhibits ACTH release in the majority of cultures of corticotrophic cells, whereas, in vivo, dopaminergic agents display an inhibitory effect on cortisol levels in a subset of patients with CS. In animal models the receptor profile can be deeply modulated in specific environmental conditions, that may resemble the different clinical phases of CS. The new insights about DRs and receptor-targeting drugs may offer different approaches for medical treatment of CS: combination therapies with different types of compounds, treatment with novel molecules (hybrid compounds) with a wider spectrum of activity, or even pretreatment manipulation of receptor profile. Finally, recent studies showed that D2R is also significantly expressed in ectopic ACTH-secreting tumors and in both normal and tumoral adrenal tissues. Dopamine-agonists may decrease cortisol levels in a number of these patients, strengthening the current (re)emerging interest in DRs as possible targets for medical treatment of CS.

It is well known that the dopaminergic pathways are crucial in the central nervous system, but dopamine plays a key role in the periphery as well, including the endocrine glands. Indeed, dopamine receptors (DRs) are involved in the regulation of the hypothalamus-pituitary-adrenal axis, and beside the control of the hypothalamic function, are differentially expressed in subsets of pituitary, as well as adrenal cells. Dopamine may activate five different receptor subtypes, codenamed D1-5R, subdivided in D1-like (D1R, D3R) and D2-like (D2R, D3R, D4R).
groups [1]. These receptors belong to the family of G-protein coupled receptors, and the D2R exists in two different isoforms, the long (D2long) and short (D2short) [1].

DRs have been found in the anterior lobe of the pituitary gland. In rats, D2R is expressed mainly in lactotrophic cells, but also in other pituitary cell subsets (somatotrope > thyreotrope > gonadotrope cells). However, D2R expression in corticotrophic cells, initially suggested by the demonstration in AtT20 cells, a mouse pituitary corticotrope line, display a rather complicated spreading, largely debated in the last decades. Similarly, in humans D2R is expressed mainly in lactotrophic cells, and in more than 75% of cells of the anterior pituitary gland, indicating a broader and complex spectrum of expression extended to non-lactotrophic cell populations. In fact, DRs, and in particular D2R, have been clearly demonstrated in the ‘intermediate lobe’ of the pituitary. Several studies in animal models (mouse, rat) have initially shown the presence of D2R in melanotropic cells, suggesting the potential expression in this cell compartment in humans as well. With respect to the isoforms, several studies on experimental models have demonstrated that both D2long and D2short variants are expressed in lactotropic and melanotropic cells, although D2long seems the predominant one. Finally, D2R is frequently accompanied by the expression of D4R, in particular its D4.4 variant, although its role in the physiology of the pituitary gland is not completely understood (fig. 1a) [1, 2]. Wu et al. [3] first demonstrated the existence of both D2R and D4R in human adrenal gland and different types of adrenal tumors, such as aldosterone-producing adenomas and pheochromocytomas (fig. 1b).

D2R is involved in the negative regulation of proopiomelanocortin (POMC) gene expression in melanotropic cells. The principal products of the POMC gene are β-endorphin and α-MSH, however, POMC in the corticotropes is principally processed into ACTH. Saiardi and Borrelli [4] demonstrated that D2R-deficient mice have increased POMC expression, intermediate lobe hypertrophy and unexpectedly elevated ACTH levels with a corresponding increase of corticosteroids and consequent hypertrophy of the adrenal gland, like in human Cushing’s syndrome (CS). Interestingly, the elevation in ACTH levels was due to an aberrant processing of POMC in melanotropes. Indeed, they demonstrated that dopamine, through its receptors, strictly regulates the expression of convertases, controlling POMC gene expression in these cells (fig. 2) [4]. These results reveal a key role for dopamine in the control of POMC-derived peptides and, furthermore, indicate an implication of the dopaminergic system in the genesis of CS.

Dopamine Receptors in Normal and Tumoral Pituitary Cells

In the recent years, the availability of new dopaminergic compounds and the new insights in the field of the dopaminergic system shed light on the pathophysiological role of dopamine and DRs, underlining their involvement in endocrine tumors, especially those arising from the hypothalamus-pituitary-adrenal axis and from the peripheral neuroendocrine system. For example, the expression and the important role of the D2R in corticotropin pituitary tumors has been clearly demonstrated. Indeed, in Cushing’s disease (CD), corticotrophic adenomas mainly express D2R and sst5, whereas sst2, the preferential target of the clinically available somatostatin analogs, is expressed in a lower rate, at least in active disease [5].

Interestingly, CD is a frequent disorder in dogs, with striking clinical similarities with CD in humans. Canine CD may, therefore, serve as an animal model for human CD, especially since therapeutic canine hypophysectomy can generate substantial amounts of primary corticotropin adenoma tissue for in vitro testing. However, De Bruin et al. have found that D2R and somatostatin receptors (SSTRs) are expressed in canine corticotrophic adenomas, but with some differences with respect to humans [6]. In particular in canine adenomas, SSTR subtype 2 (sst2) was highly expressed, probably due to glucocorticoid-induced differentially regulation, while sst5 expression was remarkably low and D2R moderately expressed. Therefore, the canine may provide an interesting model to study CD, but differences in SSTR and DR expression in comparison with humans should be taken into account when using dogs with CD as a model to evaluate the efficacy of somatostatin analogs or dopamine agonists [6].

Pivonello et al. [7], on the basis of previous studies showing the effectiveness of dopamine agonists in inducing shrinkage of silent ACTH-secreting pituitary tumors and of adenomas in Nelson’s syndrome, demonstrated the expression of functional D2R in 80% of ACTH-secreting pituitary tumors, and showed the effectiveness of a short-term treatment with cabergoline in normalizing ACTH and cortisol secretion in 40% of these patients. These data strongly support the potential therapeutic use of dopaminergic drugs in the management of persistent and/or recurrent CD. However, further studies are warranted for an accurate patients selection and possibly tumor characterization to achieve the goal of a targeted therapy in a such complex and heterogeneous disease.
Dopamine Receptors in Normal Adrenal, Adrenal Tumors and Ectopic Cushing’s Syndrome

As far as the pituitary-adrenal axis, D2R is definitively expressed in adrenal cells as well. The presence of the different DR subtypes (D1-like and D2-like families) has been described in both the normal and the hyperplastic adrenals [3, 8]. Indeed, D2R and D4R have been recorded in aldosterone- and cortisol-secreting adenomas, cortisol-secreting carcinomas, as well as clinically nonfunctioning adrenals, whereas no significant amount of DR have been detected in aldosterone- and androgen-secreting carcinomas, while D3R, D4R and D5R have been identified in pheochromocytomas. In line with these findings, and with the evidence that bromocriptine and cabergoline were effective in modulating adrenal hormones.

![Diagram showing DR expression in normal and tumoral pituitary corticotrophic cells, as well as in ectopic ACTH-producing tumors.](image-url)

**Fig. 1.** a Heterogeneity of DR expression in normal and tumoral pituitary corticotrophic cells, as well as in ectopic ACTH-producing tumors. b Heterogeneity of DR expression in different types of adrenal cells: normal cells, secreting adenoma cells, nonfunctioning adenoma and carcinoma cells. The size of each receptor indicates its quantitative expression.

Dopamine Receptors in Corticotrophic and Adrenal Cells

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production in the nontumoral adrenal gland in vitro, the DR profile in tumors suggest a potential use of dopamine agonists in adrenal disorders [8].

In ectopic ACTH syndrome (EAS) DRs, in particular D_{2}R, have been detected in neuroendocrine tumors associated with EAS [9]. In the largest study evaluating receptor patterns in EAS, the authors demonstrated that 2 patients who experienced a treatment escape or resistance were those bearing tumors expressing the long isoform of D_{2}R, associated or not with D_{4}R, whereas another patient, showing long-term responsiveness to cabergoline, harbored a carcinoid tumor expressing both isoforms of D_{2}R, as well as D_{4}R [9]. These findings suggest that the expression of the short isoform of D_{2}R, and/or the coexpression of the D_{4}R, may play a pivotal role in driving the effectiveness of DA in carcinoid tumors associated with EAS. However, further evidences are warranted to confirm this attractive hypothesis.

Another option in EAS is the possibility of adding dopaminergic agents to the treatment with somatostatin analogs. Actually, some evidence is already available in the literature [10] and is supported by the recent demonstration of a synergistic cooperation between SSTRs and DRs in different in vitro models [11]. Moreover, interestingly, as already mentioned, SSRs and D_{2}R can be differently regulated by glucocorticoids in neuroendocrine cells. This aspect should be strongly considered because cortisol lowering therapy could indirectly affect the responsiveness of tumor’s cells to SSTR- and DR-targeting drugs [11]. De Bruin et al. [12] investigated the effects of dexamethasone on D_{2}R and SSTR expression in three human neuroendocrine cell lines, BON (carcinoid) and TT (medullary thyroid carcinoma) versus DMS (small cell lung cancer), this latter severely resistant to glucocorticoids. The author demonstrated that glucocorticoids selectively downregulate sst_{2} but not D_{2}R and, in a lower rate, sst_{5} in human BON and TT cells, whereas in rodents they differentially
influence D2R and SSTR expression [12]. This mechanism may also be responsible for the low expression of sst5 in pituitary corticotropic adenomas, and underlines the current interest in drugs targeting sst5 and D2R, as potential treatment of CD. Indeed, the co-expression of DRs and SSTR has been recently evaluated in 30 patients with CD, and by quantitative RT-PCR the authors demonstrated that D2R and sst5 were co-expressed in the majority (60%) of adenomas, whereas 23% of the cases expressed only D2R [13]. The remaining 17% did not significantly express either sst5 or D2R, and adenomas with an invasive growth invariably showed the loss of expression of sst5 and D2R. The coexpression of these receptors in the majority of human corticotropic adenomas support the rationale behind sst5-D2R-targeted therapy [13, 14].

New Perspectives

Based on the fact that many neuroendocrine cells co-express both SSTRs and DRs, and that these receptors may work synergistically, probably via membrane interaction or dimerization [15], new chimeric molecules, containing both somatostatin and dopamine structural elements, have been synthesized. By binding two different receptors, these molecules may draw the receptors together, in a spatial manner, leading to a higher potency of the chimeric drugs, compared to the separate activation of SSTRs and DRs [16]. Indeed, therapy using the novel somatostatin-dopamine chimeric molecules appears to be a promising approach in this respect. In fact, a study carried on tumor fragments of GH-secreting pituitary adenomas in 22 patients, reported the comparative efficacy of octreotide, cabergoline and multiple ligands directed towards the different SSTR subtypes, such as BIM-23A779 and SOM-230, and of chimeric analogs which bind both SSTRs and the D2R, such as BIM-23A760 and BIM-23A781. The variable patterns of response to sst5, sst5 and D2R analogs may explain the greater efficacy of drugs which bind to the three receptors in suppressing GH secretion [17]. The efficacy of hybrid compounds on CD is, however, still under evaluation. The key question is to know whether or not the exposure of such multiple ligands could increase their efficacy, by comparison to the effects of the single ligands, in controlling the different pituitary adenomas. Indeed, they do not show similar effects in the control of PRL hypersecretion or in the control of cell growth in clinically nonfunctioning pituitary adenomas and lung tumor cell lines, and data about CD are not sufficient up to now [17, 18].

It was already known that dopamine (via D2R) modulates aldosterone secretion and synthesis through a specific attenuation of protein kinase C (PKC) activity, as well as the intracellular calcium level. Downregulation of the D2R in aldosterone-secreting adenoma, in turn, increases PKC activity and leads to overproduction of aldosterone in affected patients. On this basis, D2R may thus serve as a potential target for the therapy of primary aldosteronism [19].

Conclusions

The complete characterization and definition of the molecular basis of the signaling pathways involving DRs are warranted. Moreover, it is important to understand whether the inclusion of the dopaminergic affinity in the new somatostatin analogs called dopastatin could result in prolonged pharmacological properties of these molecules, or even activate alternative intracellular signaling pathways involved in the control of cell growth, as well as cell secretion [20].

In conclusion, D2Rs which are heterogeneously expressed in about 75% of ACTH-secreting pituitary tumors are functional receptors that, once activated, may produce control of the activity in almost 50% of patients with CD. D1- and D2-like receptors are expressed in normal adrenal gland. D2-like receptors are also expressed in benign and in cortisol-secreting malignant adrenal tumors. However, D2R seems mainly involved in the control of aldosterone secretion in nontumoral adrenal cells. Finally, D2R is frequently expressed in carcinoid tumors associated to ectopic CS, again linked to the control of the aberrant production of ACTH.

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