Novel insights in dopamine receptor physiology

Rosario Pivonello^{1,2}, Diego Ferone^{1,3}, Gaetano Lombardi², Annamaria Colao², Steven W J Lamberts¹ and Leo J Hofland¹

Departments of ¹Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands, ²Molecular and Clinical Endocrinology and Oncology, 'Federico II' University, Via Sergio Pansini, 5, 80131 Naples, Italy and ³Endocrinological and Metabolic Sciences, Centre of Excellence for Biomedical Research, University of Genova, Genova, Italy

(Correspondence should be addressed to R Pivonello; Email: rpivone@tin.it)

Abstract

The dopaminergic system has a pivotal role in the central nervous system but also plays important roles in the periphery, mainly in the endocrine system. Dopamine exerts its functions via five different receptors, named D₁–D₅, belonging to the category of G protein coupled membrane receptors. Dopamine receptors are heterogeneously expressed in different cells, tissues and organs, where they stimulate or inhibit different functions, including neurotransmission and hormone synthesis and secretion. In particular, the dopaminergic system has a pivotal role in the physiological regulation of the hypothalamus–pituitary–adrenal axis. Recent data have demonstrated the expression and function of dopamine receptors not only in endocrine organs but also in endocrine tumors, mainly those belonging to the hypothalamus–pituitary–adrenal axis, and also in the so-called 'neuroendocrine' tumors. These data confirm the important role of the dopaminergic system in this endocrine axis, as well as in the neuroendocrine system. This review summarizes the main structural and functional characteristics of dopamine receptors, emphasizing the most recent novelties, and focused on the physiological and pathological regulation of the hypothalamus–pituitary–adrenal axis by the dopaminergic system. In addition, the recent findings on the relationship between dopamine receptors and neuroendocrine tumors are summarized.

European Journal of Endocrinology 156 S13–S21

Introduction

Dopamine is the predominant catecholamine neurotransmitter in the human central nervous system, where it controls a variety of functions including cognition, emotion, locomotor activity, hunger and satiety, and endocrine system regulation. Dopamine also plays multiple roles in the periphery as a modulator of cardiovascular and renal function, gastrointestinal motility, and the endocrine system (1). Dopamine exerts its functions via its binding to dopamine receptors (1). In recent years, the availability of new dopaminergic compounds and new investigations into the dopaminergic system have produced novel information on the physiological role of dopamine and dopamine receptors, mainly in the endocrine system, and their implication in the management of endocrine tumors, especially regarding the hypothalamus–pituitary–adrenal axis, and the neuroendocrine system.

This paper was presented at a symposium held at the Erasmus Medical Center, Rotterdam, The Netherlands, 2005. The symposium was jointly organized by LJ Hofland, Erasmus Medical Center, and A Colao, Federico II University of Naples, Italy. Ipsen partially supported the publication of these proceedings.

Structural characteristics of dopamine receptors

Dopamine receptors belong to the family of seven transmembrane domain G protein coupled receptors and include five different receptor subtypes, named D₁–D₅. The members of the dopamine receptor family are encoded by genes localized at different chromosomal loci, displaying a considerable homology in their protein structure and function. The analysis of dopamine receptor structure and function suggests the existence of two different groups of receptors: D₁-like, including D₁ and D₅ receptors, generally associated to a stimulatory function, and D₂-like, including D₂–D₄ receptors, generally associated to an inhibitory function (1). The D₁ and D₅ receptors are encoded by genes lacking introns and share an 80% homology in their transmembrane domains. The D₂ receptor shares a 75% homology with the D₃ and a 53% homology with the D₄ transmembrane domains, and all three receptor subtypes are encoded by genes which are interrupted by introns (1). The NH₂-terminal stretch has a similar number of amino acid residues in all the receptor subtypes and carries a variable number of consensus N-glycosylation sites, which can also be present in the

extracellular loops: the D₁ and D₅ receptors possess two such sites, the D₂ has four, the D₃ has three, and the D₄ possesses only one potential *N*-glycosylation site. The carboxy terminus, which is about seven times longer for the D₁-like than for the D₂-like receptors, is rich in serine and threonine residues and contains a cysteine residue, probably involved in the anchorage of the cytoplasmic tail to the membrane. Potential phosphorylation sites are localized not only on the COOH-terminal, but also in intracellular loops, mainly the third loop. Dopamine receptors possess two cysteine residues in the second and third extracellular loops, which probably form a disulfide bridge used for the stabilization of the receptor structure. The D₁-like receptors have a short third intracellular loop, typical for receptors interacting with G-stimulatory (G_s) proteins, to stimulate cyclic AMP production, whereas the D₂-like receptors have a long third intracellular loop, typical for receptors interacting with G-inhibitory (G_i) proteins, to inhibit cyclic AMP production. The third intracellular loop is the region responsible for the G protein coupling and signal transmission. The hydrophobic transmembrane domains are responsible, through some specific amino acid residues, for the binding of dopamine as well as its agonists and antagonists (1).

The D₂ receptor exists in two main variants, the long isoform named D_{2L} and the short isoform named D_{2S}, generated by an alternative splicing of an 87 bp exon. These two D₂ receptor isoforms differ for the presence or absence of a stretch of 29 amino acid residues in the third cytoplasmic loop in their protein structure (2). The two D₂ receptor isoforms have similar pharmacological but different functional characteristics, since the third cytoplasmic loop plays a crucial role in the signal transmission, particularly in determining the G protein coupling specificity, and consequently in activating different signaling pathways (3). It is noteworthy that the two D₂ receptor isoforms have also a distinct regulation of the receptor internalization (4). Splicing variants of the D₃ receptor encoding nonfunctional proteins have also been identified. The analysis of the D₄ receptor reveals the existence of polymorphic variations within the coding sequence, being a 48 bp sequence existent as a direct repeat sequence (D_{4.1}), fourfold (D_{4.4}), sevenfold (D_{4.7}), or 11-fold (D_{4.11}) repeat

sequence. Therefore, the D₄ receptor isoforms differ for the length of the third cytoplasmic loop and have one, four, seven, or 11 times the same insert of a stretch of 16 amino acid residues in their protein structure. The D₅ receptor has two related pseudogenes, which share a 95% homology with the gene and encode for truncated nonfunctional forms of the receptor (1). The molecular characteristics of human dopamine receptor family are summarized in Table 1. A schematic representation of the human dopamine receptor is shown in Fig. 1, whereas a schematic representation of the two different D₂ receptor isoforms is shown in Fig. 2.

Pharmacological characteristics of dopamine receptors

The pharmacological profile of dopamine receptors displays a difference between D₁- and D₂-like receptors, mainly a variable binding affinity of certain dopamine agonists and antagonists (1). Dopamine binds all five receptors although among the D₁-like receptors it binds the D₁ with lower affinity than the D₅ receptor, and among the D₂-like receptors it binds the D₂ with lower affinity than D₃ and D₄ receptors. Beyond dopamine, different agonists or antagonists preferentially or exclusively bind D₁- or D₂-like receptors. For instance, among the dopamine agonists, bromocriptine preferentially binds D₂-like receptors but it is able to bind D₁-like receptors as well, whereas among the dopamine antagonists, sulpiride binds the D₂-like receptors exclusively. It is noteworthy that D₁ and D₅ receptors cannot be clearly differentiated pharmacologically, since no dopamine agonist or antagonist exclusively or preferentially binds D₁ or D₅ receptors, whereas they generally display similar binding affinities for both receptor subtypes. Conversely, the availability of dopamine agonists and/or antagonists which exclusively or preferentially bind D₂, D₃, or D₄ receptors make it possible to clearly differentiate these pharmacologically. No compound is able to clearly discriminate between D_{2L} and D_{2S} although a marginal difference in the affinities of the two D₂ receptor isoforms has been described for the dopamine antagonist sulpiride (1). Among the dopamine agonists, cabergoline is able, like bromocriptine, to bind both D₁ and D₂ receptors, with higher affinities for both receptors and higher selectivity

Table 1 Molecular characteristics of human dopamine receptors.

	D ₁ -like		D ₂ -like			
	D ₁	D ₅	D ₂		D ₃	D ₄
			D _{2short}	D _{2long}		
Amino acids	446	467	414	443	400	387–515
Amino acids in the 3rd cytoplasmic loop	57	50	134	163	120	101–261
Introns	0	0	5	6	5	3
Chromosomal localization	5q35	4p15-16	11q22-23		3q13	11q15

Figure 1 Dopamine receptor structure. Structural features of D₁-like receptors are represented. D₂-like receptors are characterized by a shorter COOH-terminal tail and by a bigger third intracellular loop. Residues involved in ligand binding are highlighted in transmembrane domains. Potential phosphorylation sites are represented on the third intracellular loop (I3) and on the COOH terminus. The potential glycosylation sites are represented on the NH₂-terminal. E1–E3, extracellular loops; 1–7, transmembrane domains; I2–I3, intracellular loops. The figure is obtained with permission from the following publication: Missale *et al.* Dopamine receptors: from structure to function. *Physiological Review* 1998 **78** 189–225 (Ref. 1).

for D₂ receptors (5). Moreover, cabergoline has recently been demonstrated to possess a higher affinity than bromocriptine not only for both D₂ receptor isoforms, but also for D₃ and D₄ receptors (6, 7). No study has ever compared the binding affinity of bromocriptine and cabergoline for the D₅ receptor. The different pharmacological characteristics of bromocriptine and cabergoline definitely demonstrate that cabergoline is more potent when compared with bromocriptine in the binding and activation of the D₂-like dopamine receptors. The pharmacological profile of the D₂-like dopamine receptors in relation to dopamine and the dopamine agonists bromocriptine and cabergoline is shown in Table 2.

Functional characteristics of dopamine receptors

Dopamine receptors mediate the effects of dopamine and dopaminergic compounds via a number of different mechanisms of signal transduction (1). Among these, the most important is the modulation of adenylyl cyclase activity resulting in either stimulation or inhibition of cyclic AMP accumulation. This mechanism is mediated via the activation of different G proteins, mainly G_{sα} for the stimulation and G_{iα} for the inhibition

of adenylyl cyclase. D₁-like receptors generally stimulate whereas D₂-like receptors inhibit adenylyl cyclase activity and cyclic AMP accumulation (1). Most commonly, the result of the stimulation or inhibition of cyclic AMP accumulation is the modulation (activation or inactivation) of protein kinase A, responsible, through phosphorylation or dephosphorylation, for the regulation of the synthesis of different cytoplasmic and nuclear proteins, function of membrane channels, and sensitization or desensitization of different G protein coupled receptors (2). Dopamine receptors are also able to activate different mechanisms of signal transduction, including the modulation of the activity of phospholipase C or the release of arachidonic acid, as well as the activity of the calcium and potassium channels. Moreover, dopamine receptors also seem to modulate the activity of Na/H exchangers and the Na–K ATPase (1). A novel mechanism of signal transmission has been recently demonstrated for the D_{2S} isoform of the D₂ receptors. The activation of this receptor is able to induce the stimulation of the phospholipase D, probably through the coupling with the Rho family of G proteins, and the involvement of a specific protein kinase C isoform (8). Phospholipase D is an enzyme able to catalyze the hydrolysis of phosphatidylcholine to phosphatidic acid and choline. The phosphatidic acid,

Figure 2 Scheme of the putative structure of the D₂ receptor isoforms. The specific amino acid stretch which is present in D_{2L} and absent in D_{2S} is indicated in the third cytoplasmic loop. Potential amino acid residues involved in glycosylation, phosphorylation and ligand binding are shown in both the D₂ isoforms. This figure is obtained with permission from the following publication: Vallone *et al.* Structure and function of dopamine receptors. *Neuroscience and Biobehavioral Reviews* 2000 **24** 125–132 (Ref. 2).

together with the diacylglycerol produced from its own dephosphorylation is an active signaling molecule implicated in a variety of cell-signaling pathways, mainly involved in the regulation of cell metabolism, as well as cell growth and differentiation. The activation of phospholipase D by D_{2S} seems to be associated with an anti-proliferative effect (9). This mechanism of signal transmission has recently been found to be associated with different dopamine receptors. It is noteworthy that dopamine receptors

are able to control cell growth and differentiation with different mechanisms (1). Recently, both D_{2S} and D_{2L} receptor isoforms have been demonstrated to activate, through the involvement of G_{βγ} subunits and protein kinase C, the mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) pathway, generally involved in the regulation of cell growth, differentiation and apoptosis (10). In particular, in pituitary tumor cell lines, dopamine agonists have been found to exert a clear anti-proliferative

Table 2 Pharmacological profile of human dopamine receptors.

	D ₂				D ₃		D ₄	
	D _{2short}		D _{2long}		E _{max} (%)	IC ₅₀ (nM)	E _{max} (%)	IC ₅₀ (nM)
	E _{max} (%)	IC ₅₀ (nM)	E _{max} (%)	IC ₅₀ (nM)				
Dopamine	100	350	100	320	100	11	100	100
Bromocriptine	41	4.5	28	3.9	68	4.2	0	> 1000
Cabergoline	102	0.53	75	0.41	86	0.78	49	81

E_{max}, maximal efficacy calculated as percentage of the maximal efficacious (100%) concentration of dopamine; IC₅₀, concentration inducing a 50% inhibition.

Figure 3 Scheme of the most important mechanisms of signal transduction associated with the activation of dopamine receptors. The figure is obtained with permission from the following publication: Missale *et al.* Dopamine receptors: from structure to function. *Physiological Reviews* 1998 **78** 189–225 (Ref. 1). The figure has been modified with the addition of some novel mechanisms discovered in the most recent years.

effect, inducing cell death and apoptosis through the D₂ receptor-mediated activation of MAPK and/or ERK pathways (11, 12). The main intracellular pathways connected with the activation of dopamine receptors are summarized in Fig. 3.

Dopamine receptors are subjected to a variety of regulatory mechanisms which can either positively or negatively modulate their expression and functional activity (13). The mechanisms underlying the different forms of dopamine receptor regulation have not yet been completely elucidated. The best information derives from studies on D₁ and D₂ receptors, which demonstrated different mechanisms of regulation although similar to the classical G protein coupled receptors. A widely studied mechanism of G protein coupled receptors is desensitization, defined as the tendency of receptor-mediated responses to wane over time despite continued stimulation of the receptor by its specific binding compound. In this process, the activation of the receptor triggers a sequence of events that result in the dampening of the receptor signal; the receptor activation promotes its phosphorylation by a member of the G protein coupled receptor kinase family leading to the binding of an arrestin-like protein, which induces an uncoupling of the receptor from its G protein as well as an internalization of the receptor through clathrin-coated pits into an endosomal compartment where it may be dephosphorylated and recycled to the cell surface or degraded via a lysosomal pathway. Several studies on dopamine receptors have demonstrated that the D₁ receptor displays a comparable mechanism of desensitization, although some difference exists from the classical scheme. In contrast, the regulation of D₂ receptors seems extremely complex with receptor activation variably resulting in functional desensitization, sensitization, up- or down-regulation (13). The regulation of D₂ receptors seems to be influenced by the type and

concentration of the agonist or antagonist, the receptor isoform and the cell system. A recent study has demonstrated that protein kinase C mediates phosphorylation, desensitization and trafficking of the D₂ receptor, and that the third intracellular loop of the receptor is mainly involved in the internalization and desensitization (14). It is important that a distinct regulation of internalization and MAPK pathway activation has been demonstrated for the two different D₂ receptor isoforms: D_{2L} activates the MAPK pathway by mobilizing growth factor receptors, probably regulated by an internalization not dependent on dynamin, a typical protein implicated in the G protein coupled receptor internalization, whereas D_{2S} appears to activate the MAPK pathway by mobilizing clathrin-mediated endocytosis and dynamin-dependent internalization (4).

Dopamine receptors, being G protein coupled receptors, are characterized by the phenomenon of oligomerization. They may form homo-oligomers, grouping with other monomers of the same receptor, or hetero-oligomers, grouping with monomers of different dopamine receptor subtypes or different receptors (15). These hetero-oligomers have functional characteristics, which are distinct from those of the single monomers or homo-oligomers, with consequent implications for receptor pharmacology, signaling and trafficking (15). The complete meaning of the homo- and hetero-oligomerization of dopamine receptors require further studies to be completely elucidated.

Distribution and role of dopamine receptors

Dopamine receptors are mainly and widely distributed in the central nervous system (1). In particular, the dopaminergic neurons in the substantia nigra, tegmental area and hypothalamus give origin to three main pathways: the nigrostriatal, the mesolimbocortical, and the tuberoinfundibular pathways. Dopamine receptors are, therefore, mainly localized in the striatum, the limbic system, the brain cortex and the infundibulum. However, the presence of dopamine receptors has been demonstrated in most areas of the central nervous system, where they mediate the effect of dopamine on cognition, emotion, regulation of hunger and satiety, locomotor activity and on the endocrine system (1). Dopamine receptors are also expressed in the pituitary gland, where they mediate the effect of dopamine on the regulation of hormone synthesis and secretion (1). Finally, dopamine receptors are widely distributed in the periphery, mainly at the level of the cardiovascular system, kidney and adrenal gland, beyond the peripheral nervous system. In the cardiovascular system, dopamine is known to induce vasodilation and increase cardiac contractility. In the kidney, dopamine induces an increase in the renal filtration rate and a decrease in salt reabsorption, as well as a stimulation of renin secretion (activating the

renin–angiotensin–aldosterone system) and inhibition of vasopressin action. The presence of dopamine receptors in the adrenal gland suggests a role for these receptors in the regulation of adrenal hormone synthesis or secretion (1).

Hypothalamus–pituitary–adrenal axis and dopamine receptors

Dopamine receptors have an important role in the regulation of the hypothalamus–pituitary–adrenal axis. Indeed, dopaminergic pathways are known to control the hypothalamic function. Moreover, increasing amounts of evidence demonstrate that the dopaminergic system also has a pivotal role in the control of the entire pituitary gland, as well as in the adrenal gland.

Dopamine receptors have been clearly demonstrated in the anterior lobe of the pituitary gland. In rats, the D₂ receptor is expressed mainly in lactotrope (16), but it is also found in non-lactotrope cells, mainly somatotrope (17) and thyrotrope (18), as well as in gonadotrope cells (19). Moreover, D₂ receptor expression in corticotrope cells has been suggested by the demonstration of its expression in AtT20 cells, a mouse pituitary corticotrope cell line (20, 21). Similarly, in humans the D₂ receptor is also expressed mainly in lactotrope cells (22), although it was found in more than 75% of cells of the anterior pituitary gland, indicating a broader spectrum of expression, not only confined in lactotrope, but also extended in non-lactotrope cell populations (23). In addition, dopamine receptors, and particularly D₂ receptors, have been clearly demonstrated in the intermediate zone of the pituitary gland. Indeed, several studies on experimental animals have shown the presence of D₂ receptors in melanotrope cells (24, 25). These data suggest the possible D₂ receptors expression in melanotrope cells in humans. With respect to D₂ receptor isoforms, several studies on experimental animals have demonstrated that both D_{2L} and D_{2S} receptor isoforms are expressed in lactotrope and melanotrope cells, although D_{2L} is predominantly expressed when compared with the D_{2S} isoform (26). Finally, the D₂ receptor is not the only dopamine receptor expressed in the pituitary gland. Indeed, the D₄ receptor, in particular its D_{4.4} variant, is also expressed, although its role in the physiology of the pituitary gland is not known (27). The role of dopamine receptors, in particular the D₂ receptor, in the pituitary gland is to mediate the regulatory effects of hypothalamic dopamine on the different pituitary cell populations. In rats and humans, the major role of the pituitary D₂ receptor is inhibitory control of prolactin synthesis and secretion, as well as the growth of lactotrope cells (22). Moreover, it has been demonstrated in rats, and hypothesized for humans, that the D₂ receptor is also involved in the inhibitory control of the synthesis and/or secretion of melanocyte-stimulating hormone and growth of the

melanotrope cells (28, 29). The finding that the expression of the Pit 1 transcription factor, which is involved in pituitary hormone gene expression, is inhibited by activation of D₂ receptors in transfected cell lines supports the existence of a dopaminergic control on pituitary hormone gene expression (30). In addition, a series of *in vitro* and/or *in vivo* studies on experimental animals and/or in humans suggests a possible modulatory role of dopamine on growth hormone, thyroid-stimulating hormone, and follicle-stimulating hormone/luteinizing hormone release from the somatotrope, thyrotrope and gonadotrope cells respectively (31). Finally, a recent study has clearly demonstrated that dopamine receptors, mainly D₂ receptors, are expressed in the majority of corticotrope pituitary tumors, where dopaminergic agents are able to inhibit adrenocorticotrophic hormone (ACTH) secretion (32). This evidence suggests a physiological role of dopamine in the regulation of ACTH release.

Dopamine receptors are also expressed in the adrenal cortex. In experimental animals, as well as in humans, receptor ligand binding studies have demonstrated the presence of specific and saturable binding sites for different radiolabeled dopaminergic compounds such as spiperone, which bind both D₁- and D₂-like receptors, and sulpiride, which selectively binds D₂-like receptors (33). These studies demonstrated that both D₁-like and D₂-like receptors are expressed in the adrenal cortex. In addition, the expression of D₁-like and D₂-like receptors in the adrenal medulla has been clearly demonstrated (34). A recent study has evaluated the expression of the D₂-like receptor subtypes in human normal adrenal glands, demonstrating that D₂ and D₄ receptors are expressed in all three zones of the adrenal cortex and in the adrenal medulla. In the cortex they are localized mainly in the zona glomerulosa, responsible for aldosterone synthesis, and zona reticularis, responsible for the synthesis of sex hormones, and to a lesser extent in the zona fasciculata of the adrenal cortex, where cortisol is synthesized (35). The expression of dopamine receptors in the adrenal cortex has been initially hypothesized after showing a role of dopamine in the control of aldosterone secretion by *in vivo* studies in both experimental animals and humans (36). The administration of the D₂ antagonist metoclopramide to both rats and humans increased the plasma aldosterone levels without influencing any stimulator of aldosterone release. This effect was blocked by the i.v. injection of dopamine (37–39). However, the administration of dopamine or the dopamine agonist bromocriptine did not modify plasma aldosterone levels (40). These observations suggest that aldosterone production was under maximum tonic dopaminergic inhibition. Subsequent studies demonstrated that the sodium balance status is crucial for the effects of dopamine or dopamine agonists on aldosterone secretion. Indeed, dopamine and D₂ agonists were shown to inhibit angiotensin-stimulated and upright posture-induced increased

aldosterone secretion in sodium-depleted but not in sodium-repleted normal subjects (41, 42). Moreover, *in vitro* studies with isolated adrenal glomerulosa cells demonstrated that the activation of D₂ receptors may result in a remarkable inhibition of angiotensin II-induced aldosterone secretion, whereas it does not influence basal and ACTH-induced aldosterone secretion (43). These studies first suggested the expression of D₂ or D₂-like receptors in the cells of the zona glomerulosa of the adrenal cortex and a selective functional interaction between dopamine and angiotensin II in the regulation of aldosterone secretion. While no definitive data are available on the role of dopamine and dopamine receptors in the adrenal cortex, their role in controlling catecholamine secretion in the adrenal medulla is well documented (44). A recent study has clearly demonstrated that different dopamine receptors, including D₁-like and D₂-like, are expressed in normal human adrenal glands, and that D₂ receptors are expressed in all three zones of the adrenal glands, although predominantly in the zona glomerulosa and zona reticularis, and in the adrenal medulla (45). In this study, dopaminergic drugs, and mainly cabergoline, showed a modulatory effect on aldosterone, but also on cortisol and androstenedione secretion, displaying a stimulatory effect at lower concentration and an inhibitory effect at higher concentrations of the compounds (45). These evidences demonstrated that dopamine may have an important physiological role in the regulation of adrenal function.

Finally, it is noteworthy that dopamine receptors have been clearly demonstrated to be expressed in corticotrope pituitary tumors (32) and in tumors deriving from the adrenal medulla, the pheochromocytomas (34, 35, 45), and have also been recently found in tumors deriving from the adrenal cortex, mainly aldosterone-secreting and cortisol-secreting tumors (45). In corticotrope pituitary tumors, D₂ receptors have an important role in mediating the inhibitory effect of dopaminergic drugs on ACTH and, consequently, cortisol secretion, so that treatment with cabergoline is able to induce remission in one-third of cases with Cushing's disease (32). Conversely, in the adrenal tumors, the role of dopamine receptors is still unknown, but the documented expression of the D₂ receptors permit a hypothesis for a possible role of dopaminergic drugs in the treatment of these tumors as well, especially in those associated to Conn's or Cushing's syndrome (45). However, no study has ever investigated the effectiveness of dopaminergic drugs in the treatment of these adrenal diseases.

Neuroendocrine tumors and dopamine receptors

Dopamine receptors have been also demonstrated in the so-called neuroendocrine tumors. Indeed, beyond

pituitary tumors, mainly including GH-secreting and PRL-secreting, ACTH-secreting and clinically nonfunctioning tumors as well as pheochromocytomas, dopamine receptors – mostly D₂ receptors – have recently been demonstrated to be expressed in a major class of well-differentiated endocrine carcinomas, known as 'carcinoids', especially those associated to ectopic ACTH syndrome, or ectopic Cushing's syndrome (46). It is possible that these receptors mediate the inhibitory effect of dopaminergic drugs in these tumors, since cabergoline has been found to induce normalization of ACTH and consequently cortisol secretion, in one out of three cases of ectopic Cushing's syndrome due to ACTH-secreting lung carcinoids (46). However, the role of dopaminergic drugs in the treatment of corticotrope ectopic tumors needs to be confirmed by further investigations on a larger number of cases of ectopic Cushing's syndrome.

Carcinoid tumors are not the only neuroendocrine tumors expressing dopamine receptors. The previous finding of dopamine receptors in gastro-entero-pancreatic cell lines (47) and the recent finding of D₂ receptors in gastro-entero-pancreatic tumors (48) suggests a role for these receptors, and possibly a role of dopaminergic drugs in this category of neuroendocrine tumors. Finally, dopamine receptors, and mainly D₂ receptors, have been also found to be expressed in a peculiar type of neuroendocrine tumors, the melanomas. However, the role of dopamine receptors in these tumors still needs to be investigated.

Conclusions

Dopamine receptors are a group of five different G protein coupled membrane receptors able to stimulate or inhibit different cell functions through the activation of inhibition of different intracellular pathways. They are heterogeneously expressed mainly in the central and peripheral nervous systems, where they mediate the effect of the neurotransmitter dopamine in several nervous functions, and in the endocrine system where they mediate the effect of dopamine on the control of hormone synthesis and secretion. In particular, the dopaminergic system has been demonstrated to be important in the regulation of the hypothalamus–pituitary–adrenal axis, which is strictly connected with the nervous system through the hypothalamus and the adrenal medulla. The dopamine receptor expression and function in the corticotrope pituitary tumors and adrenal tumors confirms the role of dopaminergic system in the hypothalamus–pituitary–adrenal axis, and suggests novel treatment strategies for Cushing's syndrome. Finally, the dopamine receptor expression in neuroendocrine tumors confirms the role of the dopaminergic system in the entire neuroendocrine system.

References

- Missale C, Nash SR, Robinson SW, Jaber M & Caron MG. Dopamine receptors: from structure to function. *Physiological Reviews* 1998 **78** 189–225.
- Vallone D, Picetti R & Borrelli E. Structure and function of dopamine receptors. *Neuroscience and Biobehavioral Reviews* 2000 **24** 125–132.
- Senogles SE, Heimert TL, Odife ER & Quasney MW. A region of the third intracellular loop of the short form of the D₂ dopamine receptor dictates Gi coupling specificity. *Journal of Biological Chemistry* 2004 **279** 1601–1606.
- Kim SJ, Kim MY, Lee EJ, Ahn YS & Baik J-H. Distinct regulation of internalization and mitogen-activated protein kinase activation by two isoforms of the dopamine D₂ receptor. *Molecular Endocrinology* 2004 **18** 640–652.
- Mantegani S, Brambilla E & Varasi M. Ergoline derivatives: receptor affinity and selectivity. *Il Farmaco* 1999 **54** 288–296.
- Newman-Tancredi A, Cussac D, Audinot V, Nicolas JP, De Ceuninck F, Boutin J-A & Millan MJ. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. Agonist and antagonist properties at subtypes of dopamine D₂-like receptor and $\alpha 1/\alpha 2$ -adrenoceptor. *Journal of Pharmacology and Experimental Therapeutics* 2002 **303** 805–814.
- Curran MP & Cabergoline Perry CM. A review of its use in the treatment of Parkinson's disease. *Drugs* 2004 **64** 2125–2141.
- Senogles SE. The D_{2S} dopamine receptor stimulates phospholipase D activity: a novel signaling pathway for dopamine. *Molecular Pharmacology* 2000 **58** 455–462.
- Senogles SE. D_{2S} dopamine receptor mediates phospholipase D and antiproliferation. *Molecular and Cellular Endocrinology* 2003 **209** 61–69.
- Choi EY, Jeong D, Park KW & Baik J-H. G protein-mediated mitogen-activated protein kinase activation by two dopamine D₂ receptors. *Biochemical and Biophysical Research Communication* 1999 **256** 33–40.
- Kanasaki H, Fukunaga K, Takahashi H, Miyazaki K & Miyamoto E. Involvement of p38 mitogen-activated protein kinase activation in bromocriptine-induced apoptosis in rat pituitary GH3 cells. *Biology of Reproduction* 2000 **62** 1486–1494.
- An JJ, Cho S-R, Jeong DW, Park KW, Ahn YS & Baik J-H. Anti-proliferative effects and cell death mediated by two isoforms of dopamine D₂ receptors in pituitary tumor cells. *Molecular and Cellular Endocrinology* 2003 **206** 49–62.
- Sibley DR & Neve KA. Regulation of dopamine receptor function and expression. In *The Dopamine Receptors*, pp 383–424. Eds KA Neve & RL Neve, Totowa, NJ: Humana Press, 1997.
- Namkung Y & Sibley DR. Protein kinase C mediates phosphorylation, desensitization, and trafficking of the D₂ dopamine receptor. *Journal of Biological Chemistry* 2004 **279** 49533–49541.
- Lee SP, O'Dowd BF & George SR. Homo- and hetero-oligomerization of G-protein-coupled receptors. *Life Sciences* 2003 **74** 173–180.
- Caron MG, Beaulieu M, Raymond V, Gagne B, Drouin J, Lefkowitz RJ & Labrie F. Dopaminergic receptors in the anterior pituitary gland. Correlation of [3H]dihydroergocryptine binding with the dopaminergic control of prolactin release. *Journal of Biological Chemistry* 1978 **253** 2244–2253.
- Cronin MJ, Thorner MO, Hellmann P & Rogol AD. Bromocriptine inhibits growth hormone release from rat pituitary cells in primary cultures. *Proceedings of the Society of Experimental and Biological Medicine* 1984 **175** 191–195.
- Foord SM, Peters JR, Dieguez C, Scanlon MF & Hall R. Dopamine receptors on intact anterior pituitary cells in culture: functional association with the inhibition of prolactin and thyrotropin. *Endocrinology* 1983 **112** 1567–1577.
- Goldsmith PC, Cronin MJ & Weiner RI. Dopamine receptor sites in the anterior pituitary. *Journal of Histochemistry and Cytochemistry* 1979 **27** 1205–1207.
- Wolfe SE & Morris SJ. Dopamine D₂ receptor isoforms expressed in AtT20 cells differentially couple to G proteins to acutely inhibit high voltage-activated calcium channels. *Journal of Neurochemistry* 1999 **73** 2375–2382.
- Wolfe SE, Howard DE, Schetz JA, Cheng CJ, Webber R, Beatty DM, Chronwall BM & Morris SJ. Dopamine D₂-receptor isoforms expressed in AtT20 cells inhibit Q-type high voltage-activated Ca²⁺ channels via a membrane-delimited pathway. *Journal of Neurochemistry* 1999 **72** 479–480.
- Lamberts SWJ & MacLoad RM. Regulation of prolactin secretion at the level of the lactotroph. *Physiological Reviews* 1990 **70** 279–318.
- Renner U, Arzberger T, Pagotto U, Leimgruber S, Uhl E, Muller A, Lange M, Weindl A & Stalla GK. Heterogeneous dopamine D₂ receptor subtype messenger ribonucleic acid expression in clinically nonfunctioning pituitary adenomas. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1368–1375.
- Lightman SL, Ninkovic M & Hunt SP. Localization of [3H]spiperone binding sites in the intermediate lobe of the rat pituitary gland. *Neuroscience Letters* 1982 **32** 99–102.
- Munemura M, Cote TE, Tsuruta K, Eskay RL & Keabian JW. The dopamine receptor in the intermediate lobe of the rat anterior pituitary gland: pharmacological characterization. *Endocrinology* 1980 **106** 1676–1683.
- Meador-Woodruff JH, Mansour A, Bunzow JR, Van Tol HHM, Watson SJ & Civelli O. Distribution of D₂ dopamine receptor mRNA in rat brain. *PNAS* 1992 **86** 7625–7628.
- Van Tol HHM, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB & Jovanovic V. Multiple dopamine D₄ receptor variants in the human population. *Nature* 1992 **358** 149–152.
- Gary KA & Chronwall BM. The onset of dopaminergic innervation during ontogeny decreases melanotrope proliferation in the intermediate lobe of the rat pituitary. *International Journal of Developmental Neuroscience* 1992 **10** 131–142.
- Desrues L, Lamacz M, Jenks BG, Vaudry H & Tonon MC. Effect of dopamine on adenylate cyclase activity, polyphosphoinositide metabolism and cytosolic calcium concentrations in frog pituitary melanotrophs. *Journal of Endocrinology* 1993 **136** 421–429.
- Elsholtz HP, Lew AM, Albert PR & Sundmark VC. Inhibitory control of PRL and Pit 1 gene promoters by dopamine. Dual signaling pathways required for D₂ receptor regulated expression of the prolactin gene. *Journal of Biological Chemistry* 1991 **266** 22919–22925.
- Tuomisto J & Mannisto P. Neurotransmitter regulation of anterior pituitary hormones. *Pharmacological Reviews* 1995 **37** 240–332.
- 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. Dopamine receptor expression and function in corticotroph pituitary tumors. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2452–2462.
- Amenta F, Chianducci L, Mancini M, Ricci A, Schena M & Veglio F. Pharmacological characterization and autoradiographic localization of dopamine receptors in the human adrenal cortex. *European Journal of Endocrinology* 1994 **131** 91–96.
- Pupilli C, Lanzillotti R, Fiorelli G, Selli C, Gomez RA, Carey RM, Serio M & Mannelli M. Dopamine D₂ receptor gene expression and binding sites in adrenal medulla and pheochromocytomas. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 56–61.
- Wu K-D, Chen Y-M, Chu T-S, Chueh S-C, Wu M-H & Bor-Shen H. Expression and localization of human dopamine D₂ and D₄ receptor mRNA in the adrenal gland, aldosterone-producing adenoma, and pheochromocytoma. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 4460–4467.
- Missale C, Lombardi C, De Cotiis R, Memo M, Carruba MO & Spano PF. Dopaminergic receptor mechanisms modulating the renin-angiotensin system and aldosterone secretion: an overview. *Journal of Cardiovascular Pharmacology* 1989 **14** (Suppl. 8) S29–S39.

- 37 Carey RM, Thorner MO & Ortt EM. Effects of metoclopramide and bromocriptine on the renin-angiotensin-aldosterone system in man: dopaminergic control of aldosterone. *Journal of Clinical Investigation* 1979 **63** 727–735.
- 38 Sowers JR, Brickman AS, Sowers DK & Berg G. Dopaminergic modulation of aldosterone secretion in man is unaffected by glucocorticoids, and angiotensin blockade. *Journal of Clinical Endocrinology and Metabolism* 1981 **52** 1078–1084.
- 39 Noth RH, McCullum RW, Contino C & Mavelik J. Tonic dopaminergic suppression of plasma aldosterone. *Journal of Clinical Endocrinology and Metabolism* 1980 **51** 64–69.
- 40 Carey RM, Thorner MO & Ortt EM. Dopaminergic inhibition of metoclopramide-induced aldosterone secretion in man: dissociation of responses to dopamine and bromocriptine. *Journal of Clinical Investigation* 1980 **66** 10–18.
- 41 Drake CR, Ragsdale NV, Kaiser DL & Carey RM. Dopaminergic suppression of angiotensin II-induced aldosterone secretion in man: differential responses during sodium loading and depletion. *Metabolism* 1984 **33** 696–702.
- 42 Malchoff CD, Hughes J, Sen S, Jackson S & Carey RM. Dopamine inhibits the aldosterone response to upright posture. *Journal of Clinical Endocrinology and Metabolism* 1986 **63** 197–201.
- 43 Missale C, Memo M, Liberini P & Spano PF. Dopamine selectively inhibits angiotensin II-induced aldosterone secretion by interacting with D₂ receptors. *Journal of Pharmacology and Experimental Therapeutics* 1988 **246** 1137–1143.
- 44 Mannelli M, Pupilli C, Fabbri G, Musante R, De Feo ML, Franchi F & Giusti G. Endogenous dopamine and DA₂ receptors: a mechanism limiting excessive sympathetic-adrenal discharge in humans. *Journal of Clinical Endocrinology and Metabolism* 1988 **66** 626–631.
- 45 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. Dopamine receptor expression and function in human normal adrenal gland and adrenal tumors. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 4493–4502.
- 46 Pivonello R, Ferone D, de Herder WW, Faggiano A, Bodei L, de Krijger RR, Lombardi G, Colao A, Lamberts SWJ & Hofland LJ. Dopamine receptor expression and function in corticotroph ectopic tumors. *Journal of Clinical Endocrinology and Metabolism* 2004 **92** 65–69.
- 47 Pivonello R, Ferone D, Lamberts SWJ & Colao A. Cabergoline plus lanreotide for ectopic Cushing's syndrome. *New England Journal of Medicine* 2005 **352** 2457–2458.
- 48 Lemmer K, Ahnert-Hilger G, Hopfner M, Hoegerle S, Faiss S, Grabowski P, Jockers-Scherubl M, Riecken EO, Zeitz M & Scherubl H. Expression of dopamine receptors and transporter in neuroendocrine gastrointestinal tumor cells. *Life Sciences* 2002 **71** 667–678.
- 49 O'Toole D, Saveanu A, Couvelard A, Gunz G, Enjalbert A, Jaquet P, Ruzsniwski P & Barlier A. The analysis of quantitative expression of somatostatin and dopamine receptors in gastro-entero-pancreatic tumours opens new therapeutic strategies. *European Journal of Endocrinology* 2006 **155** 849–857.
- 50 Bodei L, Hofland LJ, Ferone D, Mooy CM, Kros JM, Paridaens DA, Baarsma SG, Ferdeghini M, Van hagen MP, Krenning MP & Kwekkeboom DJ. In vivo and in vitro detection of dopamine d₂ receptors on uveal melanomas. *Cancer Biotherapy and Radiopharmaceuticals* 2003 **18** 895–902.

Received 28 January 2007

Accepted 30 January 2007