Evolution and cell biology of dopamine receptors in vertebrates

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Abstract

Dopamine, one of the main modulatory neurotransmitters of the nervous system acts on target cells through two classes of G protein-coupled receptors, D₁ and D₂. The two dopamine receptor classes display different structures, interact with different regulatory partners (including heterotrimeric G proteins) and, accordingly, have independent evolutionary origins. In vertebrates, each of these receptor classes comprises several subtypes, generated by two steps of gene duplications, early in vertebrate evolution. In the D₁ receptor class, the D₁A, D₁B, D₁C and D₁D subtypes, and in the D₂ class, the D₂, D₃ et D₄ receptor subtypes have been conserved in most vertebrate groups. This conservation has been driven by the acquisition, by each receptor subtype, of a small number of specific properties, which were selected for adaptive purpose in vertebrates. Among these properties, affinity for dopamine, the natural ligand, intrinsic receptor activity, and agonist-induced desensitization clearly distinguish the receptor subtypes. In addition, each dopamine receptor subtype is addressed to a specific location within neuronal networks, although detailed information is lacking for several receptor subtypes. Receptors localization at diverse subcellular places in neurons may also differ from one subtype to another, resulting in different ways of regulating cell signalisation. One challenge for future research on dopamine and its receptors would be to identify the nature of the protein partners and the molecular mechanisms involved in localizing receptors to the neuronal plasma membrane. In this respect, the evolutionary approach we have undertaken suggests that, due to gene duplications, a reasonable degree of freedom exists in the tight organisation of dopamine receptors in neurons. This “evolvability” of dopamine systems has been instrumental to adapt the vertebrate species to nearly all the possible environments.

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In Vertebrates, dopamine is a neurotransmitter acting as a modulator of neuronal activity, regulating thereby many different functions in the central nervous system. This modulatory action applies to sensory perception in the retina and olfactory bulb, regulation of prolactine release in the pituitary gland, control of body temperature, food intake and sexual behavior in the hypothalamus, tuning of sensorimotor cues in the basal ganglia. In addition, dopamine systems are central to the maintenance and expression of the qualitative values of novelty in life experiences and, thus, motivation or aversion.

The various effects of dopamine on physiological systems or organs are mediated by multiple metabotropic membrane receptors coupled to heterotrimeric G proteins (GPCR). As much as seven receptor proteins encoded by different genes have been isolated so far in vertebrates. The interest for dopamine receptors arose from the discovery of alteration in dopamine transmission in several human pathologies, essentially Parkinson’s disease, addiction to drugs of abuse, disorders of mood and affect (schizophrenia), prolactinomas of the anterior pituitary gland, hypertension or cardiovascular failure. Accordingly, dopamine receptors are the targets of major drug classes (neuroleptics, antiparkinsonian drugs, etc. (Strange, 1993; Seeman and Van Tol, 1994; Sokoloff and Schwartz, 1995).

The fact that dopamine is able to act on target cells via several different receptor molecules is common to most neurotransmitters, but the necessity for such a molecular diversity, as well as its physiological consequences are far from obvious. This is a frequently asked question in the field of molecular biology and genetics. Dopamine receptor subtypes share similar sequences, structures and functions. Ac-
cordingly, they are encoded by a gene family, the members of which have been generated by events of gene duplications, followed by selection of the duplicated genes (Vernier et al., 1995). Therefore, understanding how the genetic multiplicity of G protein coupled receptors has been created, and which is its functional counterpart, can only be achieved through an evolutionary approach (Kirschner and Gerhart, 1998). Indeed, diversification of dopamine receptors is primarily an historical phenomenon, and a better knowledge of this history is a prerequisite to any experimental analysis of the structures and functions of dopamine receptors present in different animal species. On the one hand, resemblances in gene structures highlight the conservation of fundamental functions of dopamine receptors in organisms. On the other hand, differences rely on the occurrence of discrete or large mutational events that led to modify tissue-specific gene expression and function of corresponding proteins. These differences have been selected for adaptive purpose all along evolution. Comparative analysis that brings together molecular data and adaptive physiological features is more and more necessary to build an integrated view of dopamine receptors, from molecular neurobiology to animal behaviour.

1. A brief history of the evolution of dopamine receptor genes in vertebrates

In vertebrates, dopamine receptors belong to two classes of G protein-coupled receptors. The classes of dopamine receptor were initially defined by their ability to modulate adenylyl cyclase activity and cAMP accumulation in cells (Keberian and Calne, 1979). D1 receptors are able to activate, whereas dopamine D2 receptors decrease adenylyl cyclase activity. However, it is now established that the D2 receptors are mainly responsible for modulating the activity of voltage-sensitive Ca"" and K" channels. Lately, many pharmacological compounds were used to discriminate these two receptor classes, establishing the concept of multiple receptors for dopamine (reviewed in Sokoloff and Schwartz, 1995; Missale et al., 1998).

As for most other neurotransmitter receptors, molecular cloning confirmed that D1 and D2 receptors constituted two different classes of dopamine receptors. In mammals, two receptor subtypes have been assigned to the D1 receptor class (D1A/D1 and D1B/D2), but other subtypes exist in nonmammalian vertebrates (D1C and D1D; reviewed in Kapsimali et al., 2003). Similarly, three subtypes of D2 receptors have been isolated in jawed vertebrates (D2A, D2B, D2C). The overall protein structure and topology deduced from receptor sequences reveals major differences between the two classes of dopamine receptors. The D2 receptors have a long third cytoplasmic loop and a short cytoplasmic C–terminal end, whereas D1 receptors exhibit a shorter third cytoplasmic loop but a very long C terminal tail. These protein segments are involved in coupling with heterotrimeric G protein, and accordingly, D1 and D2 receptors interact with different classes of heterotrimeric G proteins. The D1 receptors are coupled to the Gs/Golf class of Gα proteins, whereas the D2 receptors coupled essentially to G1/Ga proteins, therby modulating different intracellular signalling pathways. These facts lead to regard the two classes of dopamine receptors as very distinct, except they are both able to bind dopamine as neurotransmitter. In addition, the existence of multiple receptor subtypes, as found in vertebrates, cannot be understood without knowing more of their evolutionary origin and physiological roles.

The question of the evolutionary origin of the dopamine receptor subtypes in vertebrates can be addressed by analyzing the molecular phylogeny of the corresponding genes. When dopamine receptors are compared to other monoamine receptors (adrenergic α1, α2, β, or trace amines, or serotonergic 5HT1, 5HT2, 5HT4, 5HT5, 5HT6, 5HT7), molecular phylogenies clearly indicate that D1 and D2 receptor classes are unrelated, or at least not more closely related to each other than they are to other classes of monoamine receptors (Fig. 1). This is consistent with the distinct structural characteristics and functional properties of the D1 and D2 dopamine receptors. It implicates that they have acquired independently and convergently the ability to bind dopamine, as in the case of the other classes of monoamine receptors.

However, if molecular phylogeny highlights the structural relationships of dopamine receptors, it cannot provide, alone, robust hypotheses on the duplications and evolution of dopamine receptors in the vertebrate phylum. With this perspective in mind, the whole complement of dopamine receptors has been isolated in species belonging to groups of vertebrates diverging at different times of vertebrate evolution. The D1 receptors having no intron, all the members of the gene family can be isolated rather easily from genomic DNA. A similar approach is more difficult for the D2 receptors, the genes of which bear several large introns inside their sequence.

In jawless vertebrates (agnathans), only one D1–like sequence has been found in lampreys and in hagfish. It is thus very likely that only one type of D1 receptor exist in these species that descend from the earliest diverging vertebrates. Interestingly, these receptor sequences are not related to any of the known D1 receptor sequences from jawed vertebrates. Thus, they probably diverged from the ancestor of the vertebrate D1 receptor before the occurrence of gene duplications generating the dopamine receptor subtypes found in jawed vertebrates (gnathostomes; Fig. 1). Accordingly, from cartilaginous fish (the electric ray Torpedo marmorata) to amphibians (the toad Xenopus laevis), three different D1 receptor sequences have been found (Sugamori et al., 1994). In these species, the phylogenetic tree easily depicts three subtypes, namely, D1A, D1B and D1C. In teleost fish (European eel, puffer fish, medaka, zebra fish) extra D1 receptor sequences are found (Cardinad et al., 1997), probably corresponding to gene duplication events that occurred specifically in teleost fish (Aparicio, 2000).

In amniotes, the situation is even more complicated (Fig. 1). A fourth D1 receptor-related sequence has been
Fig. 1. A phylogenetic tree of the monoamine receptor classes and hypothesis on the evolution of dopamine receptors in vertebrates. On the left part of the figure, the sequence relationships of dopamine receptor classes (D₁ and D₂) have been analyzed, together with those of the main other monoamine receptor classes. After sequence alignment, a distance algorithm has been used to compute identities and differences, and the corresponding tree is shown here. The tree has been rooted on the muscarinic receptor group, and branch length is proportional to the overall similarities between receptor sequences. Only one example of receptor subtypes (human in general) has been used for the serotonin 5HT₁, 5HT₂, 5HT₄ receptor classes (green boxes), the class of trace amine receptors (TA; blue box) as well as for the adrenergic α₁, α₂ and β receptors (yellow boxes). The tree clearly shows that each class of monoamine receptor is very distantly related to each other (including the D₁ and D₂ receptor class), suggesting a very ancient origin, long before the emergence of vertebrates. For the dopamine receptors, the subtypes found in the various vertebrate phylum have been added, highlighting the presence of four subtypes in the D₁ class and three subtypes in the D₂ class, overall. Hypothesis on the evolution of dopamine receptors drawn from the phylogenetic analysis is shown on the right part of the figure. Two steps of gene duplications (1 and 2 in the figure), which occurred between the divergence of jawless vertebrates (agnathans) and the emergence of jawed vertebrates (gnathostomes), generated the four D₁ receptor paralogues (see text for details). Although it is very likely that a similar situation happened for the D₂ receptors, no strong evidence is available yet. After gene duplications, the dopamine receptor subtypes acquired new functional characteristics (dopamine affinity, square; intrinsic activity, circle; desensitization time course, triangle) that contributes to the conservation of the corresponding genes in the numerous species expressing them. In contrast, coupling to either Gs/olf or to Gi/o proteins are ancient properties inherited from the common ancestor of D₁ receptors, characterizing the D₁ or the D₂ receptor class. Accordingly, the agnathans D₁ receptor (Agn. D₁) also share this property with the other vertebrate D₁ receptors. Finally, the ability to bind dopamine is a convergent character of D₁ and D₂ receptor classes.
isolated from chicken and crocodiles (archosaurs) and named D1D (Demchyshyn et al., 1995). This subtype is also present in lepidosaurs (lizards and snakes). However, the third group of amniotes, turtles, expresses D1A and D1B receptor sequences, but they possess only one more sequence distinctly related to the D1C subtype. In mammals, only D1A and D1B receptor-related sequences have been isolated in eutharian (placental) mammals, as well as in metatherians (marsupials). Interestingly, in the two main species of prototherian mammals, platypus and echidnea, two D1 receptor sequences have also been found, but they are related to the D1A and D1D subtypes. The most parsimonious interpretation of these data is that four different subtypes of D1–related receptors existed in stem amniotes. Two of them have disappeared in mammals, D1B and D1C in prototherian mammals and D1C and D1D in the other groups of mammals. In other words, mammals are an exception among vertebrates since the D1C receptor subtype is present in all the other groups of jawed vertebrates, and the D1D subtype is present in all the amniotes, with the exception of placental and marsupial mammals.

A detailed analysis of the molecular phylogeny of the D2 receptor class is not possible yet. However, based on current data, the phylogenetic picture and the evolutionary history of the D2–like receptors is very similar to that of the D1 receptor class. Again, three subtypes are easily identified, (D2, D3 and D4 subtypes; Fig. 1), and they are found in most groups of jawed vertebrate. Whether one or several D2–like receptors exist in agnathans is not known at present, and we cannot write a comprehensive history of the D2 receptor class yet.

Overall, the existence of one subtype of dopamine receptor in agnathans and of two, three or four paralogous receptors in gnathostomes, supports the hypothesis of a double whole-genome duplication in the evolution of Craniates. However, it is impossible to know precisely when these duplications took place, except they should have occurred between the emergence of the earliest vertebrates and that of gnathostomes (Fig. 1). After these two gene duplications that gave birth to four dopamine receptor paralogues, several groups of species have lost one or two of these genes. For example, bony fish and amphibians have lost one D1 receptor sequence (D1D), and the mammals have lost two of these genes (D1C and either D1B or D1D).

The D1A receptor exhibits the most conserved sequence and it is the only one found in all the jawed vertebrate species. It may thus be functionally the most important of the dopamine receptors, a statement clearly confirmed by the results of specific knock-out of the D1–related genes in mouse (Sibley, 1999). In contrast, other dopamine receptor sequences are relatively more divergent (apparent “evolutionary speed”), as shown by a “long branch” in the phylogenetic tree. This is for example the case for the D2 receptor subtype in the D2 class, and for the D1C and D1D subtype in the D1 class. This “long-branch” effect conceals the “true” evolutionary relationships of the D1 receptors in vertebrates (see Fig. 1) since a fast evolving sequence will branch deep in the tree, although they have emerged late during evolution. Such a biased relationship can only be detected when the all the paralogous and orthologous genes have been isolated for a large sample of species, as it is the case for the dopamine D1 receptors.

The molecular analysis of sequence relatedness (molecular phylogenies) cannot tell why some receptor sequences have been conserved and why others have been lost. Acquisition of a novel functional character or a novel expression territory, are means by which duplicated genes gain non-redundant characters and were conserved in ancestral species. The following paragraphs give an overview of our current knowledge of the functional properties of dopamine receptors.

2. Tissue distribution and subcellular localization of dopamine receptors

A fundamental aspect of dopamine function in whole organism is the localization of dopamine receptors in the various areas of the nervous system or at the periphery of the body. From a genetic point of view, as soon as two receptors are differentially expressed in cells, they are no longer redundant, provided the cells remain submitted to the action of the transmitter. Similarly, the precise subcellular localization of receptor within the target cells are major features of receptor function, especially in highly polarized cells such as neurons. Obviously, a receptor located at a post-synaptic position on the terminal dendrites will not play the same role as a presynaptic receptor located on nerve terminals. Differential expression or localization of receptors often occurs after gene duplication, either by acquisition of “new” expression territories or by splitting the expression areas of the ancestral gene (Aparicio, 2000).

2.1. General features of dopamine receptor distribution in the vertebrate nervous system

A comparative analysis of dopamine receptors has been carried out in several important groups of vertebrates including teleost fish (Kapsimali et al., 2000), xenopus (unpublished results), birds (Schnell et al., 1999) and mammals (Tiberi et al., 1991; Meador-Woodruff et al., 1996; reviewed in Kapsimali et al., 2003). The D1 and D2 receptors are present in all of the known target areas of dopamine in the central nervous system of vertebrates and their expression territories exhibit considerable overlap. However the question about whether the different receptor subtypes are present or not in the same cells has not been definitely resolved (Gerfen, 2000). Mostly, however, neurons are not expressing simultaneously D1 and D2 receptors, or only at very different levels, revealing that the transcriptional regulation of the two dopamine receptor classes is essentially non-redundant.

The overall distribution of dopamine receptors in the central nervous system is probably conserved in many vertebrate species, since, as far as we know, the distribution pattern of
the D1 and D2 receptor subtypes is generally similar in fish, amphibians, birds, and mammals. A summary of this tissue distribution is given in Table 1. The distribution of the three, well-studied paralogous D1 and D2 receptor subtypes displays a picture of large overlap, differential abundance, with a few specific localizations. This overview indicates that, after gene duplications, the new paralogous genes have conserved, to a large extent, a similar regulation of tissue-specific expression. However, the few—but significant—differences that exist between subtypes, certainly contributed to the conservation of the duplicated genes at the origin of jawed vertebrates and were crucial in setting up many of the expression characteristics of dopamine receptors found in modern vertebrates.

Among the D1 receptors, the D1A receptor subtype is the most abundant, as well as the D2 receptor subtype in the D2 class. This holds true in all the vertebrate species examined so far. In contrast, the D1C receptor, which is not found in mammals, is only weakly expressed in the fish and amphibian brain and only in limited areas of the hypothalamus. The D1B receptor subtype is also less abundant than the D1A receptor in mammals, birds, amphibians, and fish, but it is specifically present in important functional areas such as the hippocampus. This characteristic fits well with the higher conservation of D1A sequences as compared to D1B or D1C sequences in vertebrates. Similarly, in the D2 class, the D3 and D4 receptors are by far much less abundant in the brain, as compared to D2 receptors. Moreover, the distribution of the D4 receptor transcript strikingly overlaps with that of the D2 receptor, in the dorsal striatum and cortical areas for example, suggesting a large degree of redundancy between the two receptor subtypes. Accordingly, the sequence D4 subtype is rapidly diverging, and null mutations are known in human without phenotypic consequences (Nothen et al., 1994). The D3 receptor is, on average, expressed at a lower level than the D2 receptor subtype, but it is found in areas where no or very little D2 receptors are present, such as in the islands of Calleja and in the nucleus accumbens. In this latter case, it is clearly co-express with the D1 receptor, suggesting specific interactions between the two receptors (Sokoloff et al., 2003).

These observations have important implications to understand how the various subtypes of the D1 or D2 receptors have evolved after the duplication of the ancestral genes in vertebrates. The similarities of the expression territories of the D1 receptor genes implicates that the main cis–regulatory elements controlling tissue-specific transcription of genes have been conserved after the gene duplication process. However, a few—but significant—differences are observed among regional distributions of the D1 subtypes, probably sustaining enough functional relevance in order to be conserved.

### 2.2. Subcellular localization of dopamine receptors

Recent investigations have pointed out that dopamine receptor subtypes exhibit discrete subcellular localizations...
within neuronal networks. A detailed analysis has been made for the D_{1A/D1}, the D_{1B/D5} and the D_{2} receptor subtypes only, due to the availability of good antibodies. Both D_{1A/D1} and D_{2} receptors are located at a post-synaptic location in dopamine-target neurons in the mammalian brain. For instance, electron microscopy analysis has shown that, within the pyramidal cells of the primate cortex, the D_{1} receptor is expressed predominantly on dendritic spines, whereas the D_{1B/D5} receptor is found primarily on dendritic shafts (Smiley et al., 1994; Bergson et al., 1995). In the rat striatum, D_{2} receptors are more concentrated on spiny dendrites and spine heads of the medium spiny neurons than on the plasma membrane of the somata (Levey et al., 1993). Similarly, in the olfactory bulb, the D_{2} receptor is found mainly on the distal part of dendrites and in the spines (Levey et al., 1993; Yung et al., 1995).

In addition to this typical post-synaptic location, both the D_{1A/D1} and the D_{2} receptor subtypes have been found at presynaptic sites within axon terminals, although typically not within the same projection pathway (Huang et al., 1992; Levey et al., 1993; Smiley et al., 1994). In the dopaminergic neurons of the nigrostriatal and tegmental ventral area, D_{2} receptors are highly concentrated in the distal region of dendrites and proximal part of dendrites, but also on the nerve terminals located far away from the cell bodies in the striatum. Interestingly, the mammalian D_{2} receptor exists in two isoforms generated by the alternative splicing of a small region corresponding to the third cytoplasmic loop of the receptor structure. Semi-quantitative PCR and immunohistochemical studies showed that the short D_{2S} receptor predominates in the cell bodies and projection axons of dopaminergic neurons of the mesencephalon and hypothalamus (Guivarch et al., 1995). In contrast, the long D_{2L} receptor isoform is more highly expressed in the neurons of striatum and nucleus accumbens (Khan et al., 1998), suggesting that the differential splicing of the D_{2} receptor may result in preferential subcellular localization within neurons. These studies also revealed an unusual localization of D_{2} receptors in perinuclear and other intracellular membrane compartments, probably the endoplasmic reticulum (ER).

This observation agrees with a detailed analysis of the subcellular localization of the mammalian dopamine D_{2} receptor carried out in various cell types (Prou et al. 2001). Indeed, after cell transfection, most of the newly synthesized receptors accumulate in large intracellular compartments, whereas the plasma membrane was only weakly labelled. Double-labelling experiments showed that the D_{2} receptor was mostly retained in the ER, the long isoform more strongly than the short one. This retention is accompanied by a striking vacuolization of the ER, roughly proportional to the expression levels of the two receptor isoforms. This phenomenon seems to be a consequence of the high intrinsic activity of the D_{2} receptor, promoting activation of heterotrimeric G protein inside the secretory pathway and blocking further transport in the ER. Such a mechanism may contribute to the regulation of the intracellular trafficking of the D_{2} receptors isoforms (Prou et al., 2001).

However, the mechanisms involved in transport, sorting and targeting of dopamine receptors subtypes to discrete subcellular locations remain almost entirely unknown. Recently, the proximal C–terminus of the protein sequence was shown to be crucial for the targeting of D_{1A-like} receptors to the cell surface. A four amino acid spacing of hydrophobic residues within the proximal C terminus of D_{1A/D1} and of the D_{1B/D5}, the FxxxFxxxF motif, is highly conserved among GPCR and has been suggested to interact with caveolin (Bermak et al., 2001). All three F residues within this motif are required for normal D_{1} targeting to the plasma membrane. In fact, the single substitution of any of the F to A resulted in improper processing and trapping of the mutated receptor in the ER. The membrane-bound protein DRiP78, which is specifically localized in the ER and physically interacts with the FxxxFxxxF motif, is able to regulate transport of the D_{1} receptor from the endoplasmic reticulum to the cell surface (Bermak et al., 2001). Whether similar mechanism is used for the D_{1B/D2} receptor is not known. However, it was found, that N–linked glycosylation, which takes place in the ER, is required for the correct membrane targeting of D_{1B/D2} receptor, but not for the D_{1A/D1} subtype (Karpa et al., 1999). Interaction between the γ subunit of COP-I coatomer and the C–terminus of D_{1} receptor has also been established, suggesting that this interactions may have a role in receptor transport (Bermak et al., 2002). Finally, the D_{1} receptors have been shown to interact with neurofilament-M, these latter being able to reduce the number of receptors in the plasma membrane and to decrease agonist-induced desensitization of the receptor (Kim et al., 2002).

A few laboratories have undertaken two-hybrid screens in yeast to identify dopamine receptor-interacting proteins that may regulate intracellular trafficking of the dopamine receptors subtypes. The D_{2} receptors have now been shown to interact with several proteins, such as spinophilin (Smith et al., 1999), filamin-A (Lin et al., 2001), actin binding protein ABP-280 (Li et al., 2000) and protein 4.1 N (Binda et al., 2002; Kabbani et al., 2002). These proteins all are cytoskeleton-associated proteins and they are specifically enriched in neurones. However, the role they have in targeting or stabilizing dopamine receptors at specific subcellular domains is not well understood yet. It is also possible that these cytoskeletal proteins may participate as scaffolding proteins in organizing the dopamine receptor-signalling complex. Almost all the experiments performed to analyze interactions between these proteins and dopamine receptors were carried out in cells lines and we do not know if these interactions also take place in neurons. The existence of highly specific structures such as neurites, postsynaptic densities or synaptic boutons, together with the absence of many protein partners present in neurons provide a level of complexity not easily mimicked in cells lines. Clearly, further studies are needed to establish these cytoskeletal proteins as functionally relevant in neuronal cells.
3. Functional properties of dopamine receptors and modulation of signalling pathways

As already stated, dopamine receptors have been divided into two groups, based on ligand affinity and specificity and effector coupling. Differences in ligand binding affinity, basal activity, efficacy and potency for G protein activation, or desensitization mechanisms may be expected to be derived characters that drove conservation of the dopamine receptor subtypes during vertebrate evolution. These derived characters would be in fact the discriminating properties of receptor subtypes found in modern vertebrate species. Here we show that it is indeed the case. A detailed comparison of the functional characteristics of dopamine receptors among species is only available for the D1 receptor class, but some inferences can also be proposed for the D2 receptors.

3.1. D1 receptor signalling

Receptors of the D1 class, D1A/D1 and D1B/D5, activate adenylyl cyclase through the G protein Gα in most tissues, or Gα in the striatum and the olfactory bulb (Monsma et al., 1990; Tiberi et al., 1991; Herve et al., 1993), resulting in enhanced levels of cAMP in cells. As a consequence, cAMP-dependent protein kinase (PKA) is turned on, and induces phosphorylation of cellular protein, such as DARPP-32, ARPP-21, ARPP-16 and STEP. There is also evidence that D1B/D5 receptor couples to Gα and that D1KD1 receptor couples to Gα, thereby modulating the activity of Ca2+, K+, and Na+ channels (Sidhu, 1998; Sidhu and Niznik, 2000). Nevertheless, all D1 receptor subtypes preferentially activate Gα, the coupling to others Gα proteins depending on the nature of the Gα proteins promiscuous to the receptors, on the presence of associated proteins such as calcyon, and of the activation level of the receptor (Kimura et al., 1991; Kimura et al., 1995; Lezcano et al., 2000; Sidhu and Niznik, 2000).

One of the clearest distinctions between the three D1A, D1B and D1C subtypes is made by the relative affinity of the receptors for ligand and for the basal activity of the receptor. The binding for ligands and coupling properties of the D1B/D5 receptor differ from that of the D1A/D1 receptor by a higher constitutive agonist-independent activity, an increased affinity and potency for dopamine and other agonists, and a lower affinity for inverse agonist, these properties being highly conserved among jawed vertebrates (Tiberi and Caron, 1994; Cardinaud et al., 1997; Sugamori et al., 1998). In contrast, the D1C receptor subtype exhibits essentially the same dopamine affinity and low intrinsic activity as the D1A subtype. These characteristics could be related to the structural difference of cytoplasmic tails of each receptor since in the three D1 receptor subtypes, it contains phosphorylation motif, which are both subtype-specific and conserved in all the species studied so far. Using chimeric constructions of D1-like receptors, Jackson et al. (2000) have shown that the cytoplasmic tail of human dopamine D1-like receptors regulate the binding affinity of dopamine and interacts with the other intracellular loops of the receptor to modulate G protein-coupling and second messenger activation. In addition, studies conducted in Xenopus with chimeric D1A/D1B and D1C receptors showed that the cytoplasmic tail also played a role in the desensitization mechanisms of these D1-like receptors (Sugamori et al., 1998; see below).

3.2. D2 receptor signalling

No systematic analysis of the functional characteristics of the three D2-like receptor subtypes has been undertaken, in contrast to the D1-like receptors. However, a few general features can be highlighted for the members of this dopamine receptor class. Beside synthetic compounds, some of them being fairly specific of either the D2, the D3 or the D4 receptor, the natural agonist dopamine has a significantly higher affinity (ten-to-thirty fold) for the D3 and the D4 than for the D2 receptor subtype (Sokoloff and Schwartz, 1995).

Although the inhibition of adenylyl cyclase activity is one of the most conserved characteristics of the D2-like receptors, the D2 receptor are not able to promote inhibition of cAMP production in fibroblastic cell lines. It does so only in neuronal-like cells, revealing that specific—but unknown—intracellular components may be necessary for receptor activity (Cussac et al., 1999). All three D2-like receptors are also able to modulate the activity of voltage-gated ionic channels. The D2 receptor subtype inhibits several types of Ca2+ ionic channels through Gα and activates K+ currents through Gβζ in pituitary cells (Lledo et al., 1992). Similarly, the D3 and D4 receptor subtypes are able to inhibit L and N-types of Ca2+ currents in transfected pituitary and neuronal cell lines (Seabrook et al., 1994).

Among the other effects of the D2-like receptors that have been observed (mobilisation of intracellular calcium stores, PKC activation, release of arachidonic acid, Na+/H+ exchange, inhibition of the Na+/K+ ATPase... etc), no evidence of a direct modulation of these effectors has been demonstrated (reviewed in Missale et al., 1998). However, a direct activation of PLC (Vallar et al., 1990) or the modulation of some of the MAP-kinase pathways (Lajiness et al., 1993) are probably important but underestimated effects of the D2 receptors.

In amniotes, the D2 receptor subtype exists in two isoforms of 444 (D2L) and 415 (D2S) amino acids generated by the alternative splicing of the pre-mRNA (reviewed in Missale et al., 1998). These two proteins are identical except for the third intracytoplasmic loop, where there is an insertion of 29 amino acids in D2L, relative to D2S. The question of the physiological counterpart of these two has long been debated, and a definitive answer has not been obtained yet. We have discussed above the possibility of differential localization of the D2 receptor isoforms in neurons. At first sight, an obvious idea was that the 29 amino acid insertion might elicit differential coupling of the receptor to G proteins. As a matter of fact, in some cell lines, D2L but not D2S, couple to Gα to inhibit forskolin-stimulated adenylyl cyclase (Montmayeur et al., 1993; Liu et al., 1994; Guiramand et al., 1995).
This is not found in other cells where no discriminating properties of the two D₂ receptor isoforms could be seen (Senogles, 1994; Gharemani et al., 1999). However, the ability of the D₂ receptor isoforms to block Ca²⁺ channels seems to vary between D₂S and D₂L (Liu et al. 1994). In addition, the D₂L receptor activates K⁺ channels through PTX-sensitive Gα subunits, whereas D₂S receptor effect could depend on Gαi inactivation (Liu et al., 1996). In conclusion, some clues exist for a differential activity of the two splicing isoforms of the dopamine D₂ receptor. However, it may reflect a differential localization in microdomains of the plasma membrane, as proposed above. It is the formation of a local network of interacting proteins, regulating both transduction phenomenon as well as the dynamic of the receptors between the subcellular compartments, which ultimately determines the cellular response to dopamine binding.

3.3. Dopamine receptors oligomerization and functional protein-protein interactions

Among the molecular interactions that may be crucial for dopaminergic transmission, the possibility that dopamine receptors can form homo or heteromers shed a new light on the mechanisms of transduction and receptor regulation. Many single-transmembrane receptors including growth-factor receptors and cytokine receptors depend on dimerization for proper function. In addition, metabotropic glutamate receptors and GABA receptors obligatorily dimerize to be transported to the plasma membrane and to transduce extracellular signals through G proteins. Whether monoamine G protein-coupled receptors also undergo dimerization is still a matter of debate. In addition to signal transduction, receptor oligomerization may be a prerequisite to desensitization, endocytosis and recycling of receptors (Hébert et al., 1996). Agonist or antagonist exposure may change oligomer:monomer ratio suggesting that interconversion between monomers and oligomers may be important for biological activity (Hébert and Bouvier, 1998).

Dopamine receptors have also been shown to form dimers and higher order oligomers. Evidence has been provided for dopamine D₂ dimerization in the human and rat brain by showing discrepancy in binding parameters for different ligands. Indeed binding measurements of labelled benzamide and butyrophenone derivatives led to the hypothesis that [³H]-spiperone binds to the D₂ receptor monomers whereas [³H]-raclopride interacts with receptor dimers, since [³H]-raclopride labelled about half the number of D₂ receptor sites labelled by [³H]-spiperone (Zawarynski et al., 1998). This finding was supported by the demonstration of dimers and oligomers formation of D₁ and D₂ receptors in transfected S⁹ cells (Ng et al., 1994a; Ng et al., 1996). Indirect evidence of D₂ receptor oligomerization has been obtained by the inhibition of wild-type D₂ receptor function by truncated amino and carboxy-terminal D₂ receptor portions (Lee et al., 2000). In addition, agonist-independent and dependent oligomerization was suggested by using a biochemical method based on energy transfer from fluorescent protein-tagged D₂ receptors in close spatial proximity in living cells (Wurch et al., 2001). However, no conclusive proof has been provided yet to demonstrate that oligomerization is mandatory for dopamine receptor activity.

In addition to homodimerization, it has been suggested that dopamine receptors not only form homodimers but also heterodimers with other GPCRs or even ligand-gated ion channels. Rocheville et al. (2000) have shown by a FRET-based assay that D₂ receptor interacts with somatostatin SSTR5 receptors, resulting in enhanced functional activity. Evidence for heteromerization of other dopamine receptor subtypes has been also provided. Oligomeric D₂:D₃ receptor protein associations have been detected by co-immunoprecipitation in primate and rodent brain homogenates, as well as in rat GH3 cells stably expressing a D₃ receptor (Nimchinsky et al., 1997). Scarselli et al. (2001) used a truncated receptor approach to show that the N-terminal part of a D₂ receptor could functionally associate with C-terminal portion of a D₃ receptor. In addition, evidences exist for a direct interaction between D₁ and D₂ receptors in ventral striatum and islands of Calleja (reviewed in Sokoloff et al., 2003).

The D₁ receptors certainly make hetero-oligomers with adenosine A₁ receptors (Gines et al. 2000). The A₁ and D₁A receptors are highly co-localized and directly interact in stable lines of mouse fibroblast Ltk⁻ cells and in primary cultures of cortical neurons. This selective A₁/D₁A receptor heteromerization seems to depend upon the simultaneous activation of the two receptors. Indeed, pre-treatment of the cells by A₁ receptor agonists prevents D₁A receptor activation and desensitization (Gines et al. 2000; Le Crom et al., 2002). Another evidence of adenosine-dopamine receptor antagonism was obtained by Hillion et al. (2002), who showed that adenosine A₂A and D₂ receptors are co-localized in membranes of SH-SY5Y human neuroblastoma cells stably transfected with human D₂ receptors and in cultured striatal cells. Moreover, long-term exposure to dopamine or adenosine receptor agonists resulted in aggregation, internalization and desensitization of both A₂A and D₂ receptors (Hillion et al., 2002).

Dopamine receptors heterodimerization can also modulate trafficking and activity of ligand-gated ion channels through direct protein-protein interactions. The D₃ receptor was shown to interact with the N-methyl-D-aspartate (NMDA) receptor, mediating changes in the NMDA-dependent currents Lee et al., 2002). Two regions in the D₁ receptor carboxyl tail directly and selectively couple the subunits NR₁a and NR₂A of the NMDA receptor. One interaction inhibits NMDA receptor-gated currents, and the other attenuates NMDA receptor-mediated excitotoxicity through a PI-3 kinase-dependent pathway (Lee et al., 2002). Direct interaction was also shown between D₃ receptor of dopamine and ligand-gated ion channel GABAₐ receptor (Liu et al., 2000). GABAₐ–ligand–gated channels complex selectively with D₄ receptors through the direct binding of the D₅ carboxy-terminal domain with the second intracellular loop.
of the γ2 subunit (short) of the GABAA receptor. This physical association makes possible reciprocal inhibitory interactions between these receptors.

These data highlight new mechanisms of signal transduction regulation that increase the dynamic of receptor regulation and functioning at synapses in the central nervous system, independently of classically defined second-messenger systems. Thus, it is probable that, in a near future careful examination of the dopamine receptor homo et heterooligomerization will lead to a better understanding of the specificity and diversity of dopaminergic signalling. The long-separated fields of receptor transduction and receptor localization are clearly merging now, to provide a more unitary view of receptor function.

4. Regulation of dopamine receptor activity

As other G-protein-coupled receptors, dopamine receptors undergo a variety of regulatory changes, among which agonist-induced desensitization and internalization are the most important. Desensitization and internalization are the two faces of a similar process that tends to diminish cell responsiveness to the presence of the neurotransmitter in the cell vicinity. Although receptor internalization into endocytic vesicles may be the first step of a catalytic process leading ultimately to receptor degradation, it is also part of a recycling process (Fig. 2) involved in receptor trafficking and localization at appropriate sites in the cells. Previous studies of dopamine receptors have revealed a great variability of agonist-induced desensitization subtypes.

4.1. Regulation of D1 receptor activity

The D1-like receptors undergo multiple levels of regulation after exposure to agonists. In the short term, agonists promote rapid functional uncoupling of the D1 receptor from G protein, which rely on receptor phosphorylation and palmitoylation (Gardner et al., 2001). The phosphorylation of D1 receptor is strictly dependent upon agonist occupancy/
activation of the receptor (Gardner et al., 2001). The second step consists in agonist-induced redistribution of surface receptor from a diffuse pattern of distribution to aggregates. Then, internalization of the ligand-receptor complex takes place within small vesicles in the cytoplasm (Trogadis et al., 1995). The molecular mechanism of G protein coupled – receptor internalization has not been fully elucidated, but several lines of evidence indicate that protein-kinase A, G protein-coupled receptors kinases (GRKs), arrestin, as well as clathrin and dynamin are involved (Fig. 2). As mentioned above, desensitization and internalization of D1-like receptors may represent two distinct – although interconnected – biochemical processes. For example, in SF9 cells, pretreatment with concavalin A or sucrose completely blocked agonist-induced internalization but not desensitization (Ng et al., 1995).

The respective roles of the different protein kinases, GRKs or second messenger-regulated kinases in the agonist-induced desensitization of D1 receptor is controversial. While some authors have underlined the central implication of GRKs (see Ng et al., 1994a; Tiberi et al., 1996), others have clearly shown that PKA may also play a role (Zhou et al., 1997; reviewed in Kapsimali et al., 2003). We have proposed that the differential kinetics of desensitization between D1A and DIB receptors may be due to constitutive activity of the DIB receptor, this latter favoring a “constitutive” desensitization of the receptor. The desensitization of the DIB/D3 receptor is functionally coupled to endocytosis, as for the D1A/D1 receptor (unpublished observations), but details of the process need to be investigated further.

In sharp contrast with the two other subtypes of dopamine D1 receptors, D1C receptors from fish and amphibians are neither able to desensitize, nor to be internalized. Thus, although it still needs to be fully proven, it is very probable that the lack of desensitization is an important discriminating property of the D1C receptors. To conclude, desensitization response to agonist exposure has been clearly diversified in the different D1 receptor subtypes after gene duplications in early vertebrates, and this property has probably contributed to the conservation of these subtypes later in evolution.

4.2. Regulation of receptor activity in the D2 class

The regulation of the D2-like receptors is rather different from that of the D1-like receptors and remains highly controversial. For the D2 receptor subtype, some authors have described desensitization of D2 receptors as a process closely correlated with agonist occupancy (Barton et al., 1991), and somehow related to receptor sequestration (Itokawa et al., 1996). Others have reported that D2 receptor desensitization occurred, but slowly and only after prolonged agonist exposure, with some differences between the two D2L and D2S receptor isoforms (Zhang et al., 1994). Finally and very surprisingly, an up regulation of D2 receptors promoted by agonist stimulation on cultured cells has also been shown (Filtz et al., 1993; Starr et al., 1995; Ng et al., 1997). Reasons for such discrepancies are not clear at all, except to say that the regulation of the D2 receptor subtype may have some peculiarity, perhaps the requirement of specific cell components, which is not easily recapitulated in heterologous transfected cells.

Among eccentricities of the D3 receptor subtype, a high intrinsic activity has been described (Gardner et al., 1996; Prou et al., 2001) that may promote a constitutive endocytosis and recycling of the receptor at the cell surface (Vickery and von Zawrow, 1999; Prou et al., 2001). Nevertheless, early steps of D2 receptor internalization seem to involve a rather common dynamin-dependent mechanism of endocytosis in early endosomes (Vickery and von Zawrow, 1999, Iwata et al., 1999). Interestingly, in COS-7 and HEK-293 cells the D2 receptor sequestration induced by agonist treatment is observed only after coexpression with GRK2 or GRK5 (Ito et al., 1999; Iwata et al., 1999), but not in SF9 cells (Ng et al., 1994b).

Very few studies have carefully compared the desensitization rate and mechanisms of the D2-like receptor subtypes. However, it has recently been shown that D3 and D2 receptors exhibit some differences in both desensitization and trafficking properties (Kim et al., 2001). Contrasting with the D2 receptor, the D3 subtype is not phosphorylated upon agonist exposure, in the absence of GRK. Overexpression of GRK caused only a subtle increase in the level of phosphorylation and the D3 receptor seems to have a reduced ability to translocate β-arrestin to the plasma membrane, as compared to the D2 receptor. Thus, as for the D1-like receptors, the D2 receptor subtypes are likely to be distinguishable by their
properties of regulation in cells, the mechanisms of which are also tightly linked to the intracellular trafficking and subcellular localization of the receptors.

5. Conclusions

Despite many uncertainties on how dopamine receptors are targeted to the plasma membrane and regulated in situ, the evolutionary approach to dopamine receptors has brought to light several important information on the role played by the receptors in vertebrate organisms (Fig. 3). Firstly, although no major changes in expression territories have been evidenced between the dopamine receptor subtypes in vertebrates, there are sufficient differences to rationalize the conservation of both the D1A/D1 and the D1B/D5 subtypes in most jawed vertebrates. Both in mammals and fish, the tissue distribution of D1B/D5 receptors overlap with that of the D1A/D1 receptors, but the D1B/D5 receptor subtype is the only one present in the hippocampal areas, a fact significant enough to justify the conservation of the D1B Receptor subtype. Conversely, the D1C receptor mRNA that has a highly restricted distribution in the fish brain being present mainly in the dorsal and medial hypothalamic nuclei and ventral habenula (Kapsimali et al., 2000). The precise role of these neuronal nuclei is still poorly known, but they have, for most of them no obvious homologues in mammals. This suggests that the role of the D1C receptor is related to adaptive neuroendocrine functions in fish, amphibians and most amniotes, functions that have been lost in mammals, together with the D1C receptors.

Other physiologically-relevant observations came out of our studies. The subcellular localization of D1A/D1 receptors at the basis of the dendritic spines, in close proximity to the dopamine release sites in the mammalian basal ganglia, should be related to its “low” affinity and fast desensitization rate. The D1A receptor is thus mainly a “synaptic” receptor and it is the most highly expressed in the brain (Fig. 3). At the cellular level, D1B/D5 receptors are found on dendritic shafts, far from synapses. This localization may account for the high affinity and high intrinsic activity that characterized the vertebrate D1B/D5 receptors, fitting with a “non-synaptic” neurohormonal type of receptor. In contrast, the D1C receptor subtype is resistant to desensitization, a property compatible with a regulatory role in cells permanently submitted to dopamine influence, as might be the case in the periventricular hypothalamic areas where the receptors (Fig. 3) are present. Similar suggestions can be made for the D2 receptor subtypes with the D2 subtype also being closer to the dopamine release sites than the D3 receptor, this latter playing a central role at the end of the development of the nervous system and during adaptation of the limbic areas to environmental changes.

Thus, the molecular properties of each of the receptor subtypes could account for the conservation of the duplicated dopamine receptor subtypes. Each of the D1 receptor subtypes has indeed distinct functional properties that have obvious adaptive consequences for the modulation of neuronal activity in regulatory networks. Taken together, these data suggest that the duplication of dopamine receptor genes in vertebrates has given some degree of freedom to species for adapting the morphological and functional organisation of their nervous system to various environments and behaviour. In particular, it has allowed areas controlled by dopamine, such as the basal ganglia and frontal cortex to undergo large quantitative changes, especially in mammals, without dramatically modifying the general properties of the dopamine systems. Thus, as very often during evolution, significant physiological changes have been reached at low genetic costs.
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References


