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# Pharmacological and Molecular Basis for Dopamine D-2 Receptor Diversity

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## Contents

## Abstract

This review will focus on the main lines of evidence that suggest the existence of multiple types of dopamine D-2 receptors.

Dopamine D-2 receptors share structural elements suggesting that they belong to a gene superfamily classified as G-protein-coupled receptors and show an archetypical topology predicted to consist of seven putative transmembrane domains. Activation of D-2 receptors results in a variety of responses, including inhibition of cyclic AMP formation, inhibition of phosphoinositol turnover, increase of K-channel activity, and inhibition of Ca influx. The G protein(s) linking the D-2 receptors to these responses have not been completely identified, nor has the possible hierarchy of these regulatory proteins in transforming the incoming signal into a change of second-messenger levels. A lot of experimental data support the hypothesis that there are multiple signal-processing pathways activated by dopamine through D-2-receptor stimulation. Recently, the identification of dopaminergic drugs that discriminate among the different transduction pathways and the isolation of distinct cDNAs encoding proteins that share binding profile indicative of D-2 receptors clearly indicate multiple forms of D-2 receptors.

Pharmacologically, at least two distinct categories of dopamine D-2 receptors exist in rat pituitary. The first (D-2a) is insensitive to BHT 920 and coupled to inhibition of adenylyl cyclase activity; the second (D-2b) is activated by BHT 920 and linked to voltage-dependent K channels. The two types of dopamine D-2 receptors differ in their structure, G-protein-coupled and effector. Each of the three basic receptor units shows a certain degree of heterogeneity, which may affect the quality and the kinetic of the response. This variety may represent the molecular basis for the diversity in pharmacological and functional profiles of different dopamine D-2 receptors located in various brain areas and peripheral tissues.


**Index Entries:** Dopamine; D-2 receptor; second messengers; potassium channels; gene expression.

## Introduction

Specific cell-surface receptors bind circulating or synaptic dopamine as the first step in mediating a variety of intracellular effector functions. Receptors for dopamine have been distinguished as D-1 and D-2 according to the types of signal transduction pathways they stimulate and the sensitivity to different specific dopaminergic agonists and antagonists (Spano et al., 1978; Kebabian and Calne, 1979). The major impetus and the most convincing evidence for the existence of "multiple dopamine receptors" resulted from the availability of agonist and antagonist molecules capable of discriminating between the two categories of receptors. Using these drugs as a tool, it was possible to clearly define structural and functional differences between D-1 and D-2 receptors.

Pharmacologically, D-1 receptors are classified as those receptors selectively stimulated by dopaminergic agonists, such as fenoldopam and SKF 38393 (Setler et al., 1978), and selectively blocked by dopaminergic antagonists, such as SCH 23390 (Hyttel, 1983). On the other side, D-2-receptor-selective agonists are quinpirole (Tsuruta et al., 1981), Ru 24213 (Euvrard et al., 1980), and various dopaminergic ergot derivatives (Trabucchi et al., 1976), whereas D-2-receptor-selective antagonists are sulpiride and other substituted benzamides (Spano et al., 1979).

Functionally, dopamine D-1 receptors act via stimulation of adenylyl cyclase activity (Kebabian et al., 1972), whereas D-2 receptors are either inhibitory or not coupled to this enzyme (Garau et al., 1978; Schwarcz et al., 1978). The pharmacological and functional profiles of D-1 and D-2 receptors have been extensively reviewed (Creese et al., 1983; Memo et al., 1986a; Seeman and Grigoriadis, 1987; Vallar and Meldolesi, 1989; Andersen et al., 1990).

Dopamine-receptor subtypes are also differentially distributed among cerebral neurons and endocrine cells (Boyson et al., 1986). For example,

neurons originating from substantia nigra, pars compacta, and projecting to caudate putamen appear to express D-2, but not D-1, receptors (Spano et al., 1977; Meador-Woodruff et al., 1989; Le Moine et al., 1990; Dearry et al., 1990). Lactotrophs also contain exclusively dopamine receptors of the D-2 type (Caron et al., 1978). Finally, D-1, but not D-2, receptors are present in parathyroid cells (Attie et al., 1980; Sunahara et al., 1990).

Recent investigations in a variety of disciplines have suggested that the D-1/D-2 classification scheme is no longer adequate to account for all experimental findings regarding the action of dopamine and various dopaminergic drugs. Recent data are consistent with a certain degree of heterogeneity of D-1 receptors (Felder et al., 1989; Mahan et al., 1990). This review will focus on the main lines of evidence that suggest the existence of multiple types of dopamine D-2 receptors.

## Heterogeneity in the Transduction Pathways Activated by Dopamine D-2 Receptors

The D-2 receptor was initially described as the receptor that is not coupled, or is perhaps coupled in an inhibitory fashion, to adenylyl cyclase. It now appears that both possibilities hold true.

### *Inhibition of Adenylyl Cyclase Activity*

Inhibition of adenylyl cyclase was first demonstrated to be coupled to the activation of D-2 receptor in a prolactinoma broken-cell preparation (De Camilli et al., 1979). Later, the dopamine-inhibition of adenylyl cyclase activity was found in membrane preparations from pituitary and various dopaminergic brain regions (Giannattasio et al., 1981; Stoof and Kebabian, 1981; Enjalbert and Bockaert, 1983). This effect could be mimicked by various D-2-selective agonists, such as quinpirole and bromocriptine, and specifically blocked by D-2-receptor antagonists, such as (–)sulpiride. Particularly, dopamine and various dopaminergic agonists are able to inhibit both basal and stimulated adenylyl cyclase activity (Munemura et al., 1980; Enjalbert et al., 1986). For example, the stimulation of enzyme activity elicited by vasoactive intestinal peptide in pituitary cells or striatal neurons in culture was specifically inhibited by dopamine (Onali et al., 1981; Weiss et al., 1985). Paradoxically, dopamine, through D-2-receptor stimulation, inhibited the increase in formation of cAMP induced by dopamine D-1-receptor stimulation (Stoof and Kebabian, 1981). This modulatory effect has been described in striatum, but not in nucleus accumbens (Stoof and Verheijden, 1986). These observations are indicative of a prominent role of D2 receptors in the "receptor–receptor" interaction and suggested that dopamine may serve as an inhibitory modulator of many different adenylyl cyclase-linked neurotransmitter receptors.

Inhibition of adenylyl cyclase activity was believed to be the sole transduction mechanism coupled with D-2 receptors, and measurement of adenylyl cyclase activity in response to D-2-receptor agonists became a paradigm to detect changes in receptor number and function (Memo et al., 1987). Using this approach, it was evident that multiplicity and greater complexity of the D-2 receptor signaling events are present. As an example, it was found that necrosis of striatal interneurons induced by kainic acid results in a disappearance of D-2-receptor-mediated inhibition of adenylyl cyclase activity associated with a reduction of about 50% of the dopaminergic binding sites as labeled by tritiated sulpiride (Memo et al., 1986b). The presence of D-2 receptors not linked with inhibition of adenylyl cyclase activity was also suggested by studying the molecular mechanisms by which dopamine modulates the release of dopamine itself from nerve terminals of the nigrostriatal pathways through stimulation of putative autoreceptors (Bowyer and Weiner, 1989).

## Inhibition of Calcium Influx

In 1985 we proposed that D-2 receptors may be coupled, at least in part, to transduction mechanisms different from adenylyl cyclase inhibition (Memo et al., 1985). Originally, we found that dopamine and various dopaminergic drugs inhibit both basal and neurotensin-stimulated calcium influx into rat lactotrophs. This effect was pharmacologically characterized as D-2-receptor mediated (Memo et al., 1986c). The possibility that reduced calcium permeability induced by dopamine was not a consequence of a decrease in the formation of cAMP was tested by two different experimental approaches. First, intracellular cAMP levels were measured in lactotrophs exposed for 5 min to very low concentrations of dopamine (1–10 n*M*). These experimental conditions allow dopamine to produce a nearly maximal inhibition of neurotensin-induced calcium influx without affecting intracellular cAMP concentrations (Memo et al., 1986c). The second approach was to examine the effects of dopamine in lactotrophs pretreated with different concentrations of pertussis toxin. The exposure of target cells, such as lactotrophs, to pertussis toxin can abrogate the inhibitory mechanisms controlling the cyclase system. In particular, the ability of pertussis toxin to abolish the inhibitory influence of dopamine receptors on adenylyl cyclase activity has been attributed to the NAD-dependent, pertussis-toxin-catalyzed, ADP rybosylation of the 41-kDa subunit of the cyclase-inhibitory G protein ($G_i$) (Dolphin, 1987). When the cells were pretreated with 100 ng of pertussis toxin, dopamine, as expected, lost its ability to decrease adenylyl cyclase activity, whereas it maintained the property of inhibiting neurotensin-stimulated calcium influx. Interestingly, a supramaximal concentration of pertussis toxin (1 μg) impaired the ability of dopamine to reverse the neurotensin effects, indicating that the dopamine receptors mediating inhibition of calcium influx are coupled to a pertussis-toxin-sensitive G protein that is different from the $G_i$ (Memo et al., 1988).

## Inhibition of Phosphatidylinositol Turnover

Inhibition of polyphosphoinositide hydrolysis has been suggested as an additional transducing mechanism elicited by D-2 receptor stimulation. This hypothesis was supported by data obtained in intact pituitary cells or in striatal slices showing that activation of dopamine D-2 receptor results in a decrease of basal or Thyrotropin Releasing Hormone (TRH) induced inositol phosphate content (Simmond and Strange, 1985; Enjalbert et al., 1986; Pizzi et al., 1988). More recent data, however, showed that in isolated membranes from lactotrophs dopamine was unable to modify both basal and TRH-induced inositol phosphate accumulation (Vallar et al., 1988). The current view is that the inhibition of inositol phosphate production induced by D-2 receptor stimulation is a late and calcium-dependent effect, possibly secondary to an inhibition of calcium entry into the cells. This hypothesis is supported by the observation of a delay in the response and an absolute requirement of calcium (Vallar et al., 1988).

## Activation of Potassium Channels

We thus have been interested in investigating the molecular mechanisms responsible for the inhibition of calcium influx elicited by dopamine. Among various possibilities, dopamine may inhibit calcium influx directly, acting on the heteromeric channel, or indirectly, by opening K channels. The latter has been shown to be one of the transduction mechanisms elicited by somatostatin (Luini et al., 1986). To test this hypothesis, a study was designed to assess both the presence of the K channels and their sensitivity to ions and drugs in the plasma membrane of rat lactotrophs. Radioactive rubidium (Rb) efflux was used to measure K permeability (Bartschat and Blaustein, 1985). We reported the existence of at least two different K channels that participate differently in the regulation of K fluxes under both resting and stimulated conditions

(Castelletti et al., 1989). The two different components of K fluxes were found on the basis of their kinetic properties and their sensitivity to both calcium and various K-channel blockers. Together with some electrophysiological observations (Petersen and Muruyama, 1984; Ingram et al., 1986; Blatz and Magleby, 1987; Cobbett et al., 1987), our data suggested that in lactotrophs a relatively slowly activated, calcium-insensitive, and voltage-dependent (voltage-operated) K channel is involved in regulating the resting membrane potential. On the other hand, a more rapidly activated, calcium-dependent (calcium-activated) outward current may play an important role in controlling membrane repolarization. Dopamine appears to affect both types of K-efflux components.

### Voltage-Regulated K Channels

Dopamine increases the efflux of Rb in a nominally calcium-free medium. This effect was statistically significant 15 s after the exposure of the cells to dopamine and was pharmacologically characterized as mediated by D-2 receptor. The enhancement of Rb efflux induced by dopamine, which reflects an increased K outward flow, can be correlated to the well-known ability of this amine to hyperpolarize the cell membrane. Increasing the concentrations of K to gradually depolarize the cells caused the increase in Rb efflux induced by dopamine to be apparently faster, evident in the initial 2–5 s of incubation. These data suggest that dopamine activation of voltage-regulated K channels is sensitive to the resting membrane potential values.

Treatment of the cells with the adenylyl cyclase activator forskolin left unchanged the ability of dopamine to increase K permeability through the voltage-operated K channels, indicating that the dopamine-mediated activation of voltage-regulated K channels is totally independent of intracellular cAMP concentrations. This is further supported by data showing that the hyperpolarizing effects, as well as the inhibition of calcium influx, induced by dopamine are unaffected by different cAMP-elevating agents (De Viegler et al., 1986; Malgaroli et al., 1987). In summary, dopamine may, independently of inhibiting cAMP formation, directly affect the functional state of a voltage-activated K channel, allowing an increase in K permeability that may result in an inhibition of calcium influx. However, the support of direct electrophysiological experiments with patch-clamp technology is needed.

### Calcium-Activated K Channels

Dopamine was also able to increase K permeability in the presence of calcium. This effect was dopaminergic in nature and completely calcium-sensitive. Interestingly, the increase of Rb efflux through calcium-activated K channels induced by dopamine was blunted by treatment of the cells with forskolin. This observation indicates that activation of the Ca-dependent K channels induced by dopamine is indirect, being mediated by a reduction in cAMP formation. It can be suggested that dopamine inhibits adenylyl cyclase activity and promotes the following series of events: reduction in the intracellular concentrations of cAMP, decrease of protein kinase A activity, and decrease in the phosphorylation state of specific subunits assembled in the calcium- activated K channels to allow K efflux.

All in all, these results indicate that dopamine affects the electrophysiological properties of pituitary cells by opening two separate K channels: a voltage-activated, calcium-insensitive, and cAMP-independent K channel and a calcium-activated, cAMP-dependent K channel (Castelletti et al., 1989). These data support the hypothesis that there are multiple signal-processing pathways activated by dopamine through D-2 receptor stimulation.

However, it is still unclear whether the various subtypes of D-2 receptor may be functional subtypes, rather than structural subtypes. A given receptor may in fact modulate via its second messenger various types of ion channels. Thus, the immediate second-messenger response might characterize the receptor function.

## Heterogeneity in the Structure of Dopamine D-2 Receptor

### *Pharmacological Evidences*

The data reported above clearly indicated multiple transduction mechanisms for D-2 receptors; however, they are not indicative of different receptor proteins. In other words, the question was whether a single D-2 receptor protein is able to promote the activation of different effectors, or whether different D-2 receptor proteins are linked to specific transduction systems. The hypothesis of heterogeneity in the D-2 receptor molecules could be elucidated by compounds specific for the different transduction systems. We found that the dopaminergic azepine derivative BHT 920 (Pichler and Kobinger, 1981; Anden et al., 1982) selectively stimulates voltage-dependent K fluxes without modifying adenylyl cyclase activity (Pizzi et al., 1990). This observation is consistent with the idea of heterogeneity in D-2 receptor molecules.

In particular, we found that the increase of prolactin secretion induced by neurotensin was inhibited by both quinpirole and BHT 920 in a sulpiride-dependent fashion. However, the release of prolactin induced by the adenylyl cyclase activator vasoactive intestinal peptide was inhibited only by quinpirole, BHT 920 being completely inactive. Contrary to quinpirole, BHT 920 was virtually unable to modify both basal and vasoactive intestinal peptide-stimulated adenylyl cyclase activity. By measuring Rb efflux as an index of K permeability on mammotroph cells, we found that quinpirole significantly activated both voltage-dependent and calcium-dependent (cAMP-sensitive) K channels. In contrast, BHT 920 induced a significant stimulation of the voltage-dependent component of K fluxes without affecting the calcium-activated K channels (Pizzi et al., 1990).

Since the presence of multiple transduction mechanisms for D-2 receptors was proposed, BHT 920 is the first agonist found to selectively stimulate one of them. Its total ineffectiveness in inhibiting adenylyl cyclase activity, as well as the cAMP-dependent prolactin release and K flux, makes BHT 920 a potentially useful pharmacological tool for the characterization of the D-2 receptors selectively linked to the activation of the voltage-dependent K channels.

Therefore, in view of their pharmacological and biochemical properties, the presence of at least two populations of dopamine D-2 receptors in rat mammotrophs can be proposed. One type, which we suggest be referred to as D-2a, represents a D-2 receptor insensitive to BHT 920, mainly coupled to inhibition of adenylyl cyclase activity; a second type, referred to as D-2b, includes receptors associated with activation of voltage-dependent K channels and selectively stimulated by BHT 920.

### *Structural Evidences*

Dopamine D-2 receptors share structural elements, suggesting that they belong to a gene superfamily classified as G-protein-coupled receptors. Analysis of the primary amino acid sequence of these receptors deduced from their cDNA or genomic clones reveals a common structural motif consisting of seven hydrophobic domains (Bunzow et al., 1988). The current model for the secondary and tertiary structures of these receptors is predicted to consist of seven membrane-spanning segments joined together by extracellular and cytoplasmic loops, with the amino terminus being extracellular and the carboxyl terminus being intracellular. The regions with greatest amino acid identity among the different members of the receptor superfamily are clustered within the putative transmembrane domains.

The glycosylated *N*-terminal segment of the receptor and the short, hydrophilic peptides connecting transmembrane-spanning segments II-III, IV-V, and VI-VII are predicted to face the extracellular surface of the plasma membrane. On the contrary, the peptides connecting transmembrane-spanning segments I-II, III-IV, and V-VI are thought to lie within the cytoplasm. The third

cytoplasmic loop, which is longer than the others and contains consensus sequences for protein kinase A phosphorylation, appears to be critical for productive receptor-coupling to G protein.

Interestingly, the gene for the D-2 receptor produces two receptor isoforms by alternative messenger-RNA splicing, providing a route to receptor diversity (Giros et al., 1989; Grandy et al., 1989; Monsma et al., 1989; Dal Toso et al., 1989, Selbie et al., 1989). One isoform encodes a receptor protein 415 amino acids long, whereas the second contains an additional sequence encoding a fragment of 29 amino acids in length. Expression of the two isoforms is tissue-specific, and both are regulated by guanyl nucleotides. The relative abundance of the mRNAs encoding the two different isoforms is not uniform in the various brain areas. The ratio between the levels of the long and the short isoforms is about 100 in pituitary, 10 in striatum, and 1 in substantia nigra (Memo, unpublished results). These observations support a distinct functional role for each individual isoform. As the extra sequence is located within the third intracellular loop that binds to G proteins, the two isoforms might interact with different G proteins and thereby initiate distinct intracellular signals. Pharmacologically, however, the two receptor forms appear to be closely related. Stimulation of each individual isoform of D-2 receptors artificially expressed in a variety of undifferentiated cell lines results in a G-protein-mediated inhibition of adenylyl cyclase activity. It will be interesting to examine in more detail whether the differential pattern of expression of the different G proteins, as well as the availability of effector systems, in the membranes of the cells used for transfection experiments may affect the functional properties of the two different isoforms of D-2 receptors (Vallar et al., 1990).

In addition to the two D-2 receptor isoforms, using rat genomic DNA it was possible to produce a transfected mouse-fibroblast cell line that expresses a novel dopamine D-2 receptor subtype. This receptor binds tritiated spiperone and possesses pharmacological characteristics of D-2 dopamine receptors (Todd et al., 1989). Functionally, stimulation of these receptors facilitates the entry of calcium into the transfected cells and increases intracellular levels of free inositol phosphates (Todd et al., 1989). These effects were not affected by pertussis toxin or cholera toxin treatment, suggesting that the expressed receptors are not linked to $G_o$, $G_i$, or $G_s$.

Finally, a dopamine receptor that differs in its pharmacology and signaling system from the D-1 or D-2 receptor and may represent both an autoreceptor and a postsysnaptic receptor has recently been characterized and cloned. This receptor has been named D-3 (Sokoloff et al., 1990). Northern blot analysis reveals high levels of mRNA encoding D-3 receptors in olfactory tubercle and nucleus accumbens. D-3 receptor signals are also detected in other limbic areas, such as the hippocampus, septum, or mammillary nuclei of the hypothalamus. Surprisingly, D-3 receptor mRNAs are not detectable in pituitary. When expressed in COS-7 cells, D-3 receptor shows a high affinity for "atypical" neuroleptics, such as sulpiride and clozapine.

Isolation of distinct cDNAs encoding proteins that share a binding profile indicative of D-2 receptors clearly indicates multiple forms of D-2 receptors. However, the definite proof that links the pharmacological and molecular evidence for D-2-receptor heterogeneity is still missing.

## Receptor Structure and Information Processing

### G-Protein-Coupled Dopamine D-2 Receptors

From the functional and structural point of view, dopamine D-2 receptors belong to the G-protein-coupled receptor family. The signal transduction pathway induced by dopamine through dopamine D-2 receptors is initiated by the binding of agonist ligands to the receptors in the plasma membrane of the cell, which stimulates the interaction of the receptors with specific G proteins.

G proteins are a family of membrane-associated proteins that are structurally homologous and widely distributed in the central nervous system (Graziano and Gilman, 1987; Neer and Clapham, 1988; Birnbaumer, 1990). Well-characterized members of the G-protein family include $G_s$ and $G_i$, which are involved in stimulation and inhibition, respectively, of hormone-sensitive adenylyl cyclase activity, and transducin ($G_t$), which is present in retina and regulates cyclic GMP phosphodiesterase activity in response to light illumination. Other members of the G-protein family, such as $G_o$, $G_x$, and $G_z$, have been identified, although their precise function has not yet been clearly established (Gilman, 1987).

G proteins are heterotrimers composed of an $\alpha$ subunit, a $\beta$ subunit, and a $\gamma$ subunit. The $\alpha$ subunit, which contains the guanine nucleotide binding site, is unique for each G protein, whereas the $\beta$ and $\gamma$ subunits are similar, if not identical. Molecular cloning techniques have allowed the isolation and characterization of cDNAs encoding several $\alpha$ subunits. The main point arising from these studies is the existence of multiple molecular species of $G_s$, $G_i$, $G_o$, and $G_t$, each having discrete individual functions (Itoh et al., 1988; Mullaney and Milligan, 1990; Mattera et al., 1989; Grunwald et al., 1986). In the case of the multiple forms of both $G_i$ and $G_s$, the primary difference is in the rates of guanine nucleotide binding and release (Graziano et al., 1989; Carty et al., 1990).

The receptor–G-protein interaction causes the release of GDP from the guanine nucleotide binding site, allowing GTP to bind (Rodbell, 1980). The GTP-bound form of the G protein, which is the activated form, dissociates from the receptor and activates the effector system, modulating the intracellular levels of specific second messengers. Thus, primary differences in the rates of guanine nucleotide binding and release may affect the function of the effector systems. In particular, an intrinsic propensity to undergo these processes would result in a greater number of G proteins activated per unit of receptor occupancy. By altering the availability of G proteins, the cell would be able to alter the kinetics of its responsiveness to extracellular signals. If rates are sufficiently different, then such differences in guanine nucleotide binding and release, and the relative availability of specific forms of G proteins, could prove crucial in regulating the extent of signal transduction. Careful documentation of the levels of the individual G proteins and correlation to the kinetics of receptor-stimulated response should yield information on whether the relative abundance of the different forms of G proteins can regulate the rate and extent of signal transduction.

Effector systems suggested as being coupled to dopamine D-2 receptors through G proteins include adenylyl cyclase and Ca and K channels. Indeed, activation of D-2 receptors results in a variety of responses, including inhibition of cAMP formation, inhibition of phosphoinositol turnover, increase of K-channel activity, and inhibition of Ca influx. The G protein(s) linking the D-2 receptors to these responses have not been completely identified, nor has the possible hierarchy of these regulatory proteins in transforming the incoming signal into a change of second-messenger levels.

The inhibition of adenylyl cyclase activity that results from activation of dopamine D-2 receptors is blocked by pertussis toxin. This observation implies the involvement of a member of the $G_i/G_o$ family. Pertussis toxin, although with different sensitivity, also inhibits the increased voltage-dependent K permeability induced by dopamine (Memo et al., 1988), suggesting that this information processing is transduced by an isoform of $G_i/G_o$ protein that is different from that mediating the inhibition of adenylyl cyclase activity. Recovery experiments using D-2 receptors partially purified from bovine anter-ior pituitary, and porcine striatal D-2 receptors reconstituted with $G_i$ and $G_o$ preparations sug-

gest that at least two different G proteins are involved (Senogles et al., 1987; Ohara et al., 1988). As in the case of many other receptors working through members of the $G_i/G_o$ family, the precise identity remains to be defined.

## Similarities with Neurotransmitter-Operated Ion Channels

Rapidly operating ion channels function as effectors for glutamate, GABA-A, glycine-1, and nicotinic receptors. Transmitter-regulated ion channels are heteropolimeric protein structures that selectively allow the passage of either anions or cations (Barnard et al., 1987; Duman et al., 1987; Costa, 1989). They are composed of 4–5 subunits with heterogeneity in their structure and diverse brain distribution (Uniwin et al., 1989; Reynolds and Karlin, 1978).

The new concept that is originating from studies on the ion-gated neurotransmitter receptor family can be summarized as follows: The receptor-subunit composition is critical for processing the information triggered by the agonist. In particular, studies on nicotinic and GABA-A receptors have established that the specificity and the quality of the response depends on the presence of a specific subunit assembled in the receptor (Schofield, 1989; Duboisin et al., 1989; Hartman and Claudio, 1990; Puia et al., 1989).

Moreover, the gene expression of individual subunits of the GABA-A or nicotinic receptors is cell-specific, suggesting that target cells are continuously regulated to express receptors with specific function and ligand sensitivity (Goldman et al., 1988; Vicini, 1991; Memo et al., 1991).

The concept of heterogeneity in receptor structure could be applied to the G-protein-coupled dopamine D-2 receptor. The proteins required to transduct a synaptic signal into a functional change inside the target cell (receptor proteins, G proteins, effector proteins) might be considered as "subunits" of a multiprotein receptor complex. Each of them can be expressed in different isoforms that carry intrinsic properties to affect the quality and the kinetics of the signal transduction.

## Expression of Dopamine D-2 Receptor Gene

The relevance of the receptor structure in dictating the quality of the dopamine response suggests that the expression of the various components of the multiprotein dopamine D-2 receptor complex may be cell-specific and neurotransmitter-regulated.

Up to now, there have been two reports showing that genes encoding D-2 receptors can be activated by exogenous factors. Y-79 human retinoblastoma cells grown in serum-free medium have been shown to undergo differentiation in response to dibutyryl cAMP. This treatment allows the cells to express very high levels of dopamine D-2 receptors (Monsma et al., 1990). Furthermore, we have demonstrated that exposure of GH3 cells to epidermal growth factor for 4 d causes the cells to express functional dopamine D-2 receptors (Missale et al., 1990).

On the other hand, it has been shown that chemical and pharmacological deafferentation causes elevation in the expression of specific mRNAs encoding D-2 dopamine receptors, which is reversed by subsequent continuous treatment with the D-2 agonist quinpirole (Le Moine et al., 1990; Gerfen et al., 1990).

These observations suggest that specific factors, possibly coming from surrounding cells, such as glial cells and/or afferent neurons, may regulate gene expression and commit the target neurons to select a range of receptor proteins for optimizing the elaboration of the dopamine signal. This process may be operative during development and/or adaptation to abnormal stimuli (receptor up- and downregulation), as well as after pharmacological treatments. This is sup-

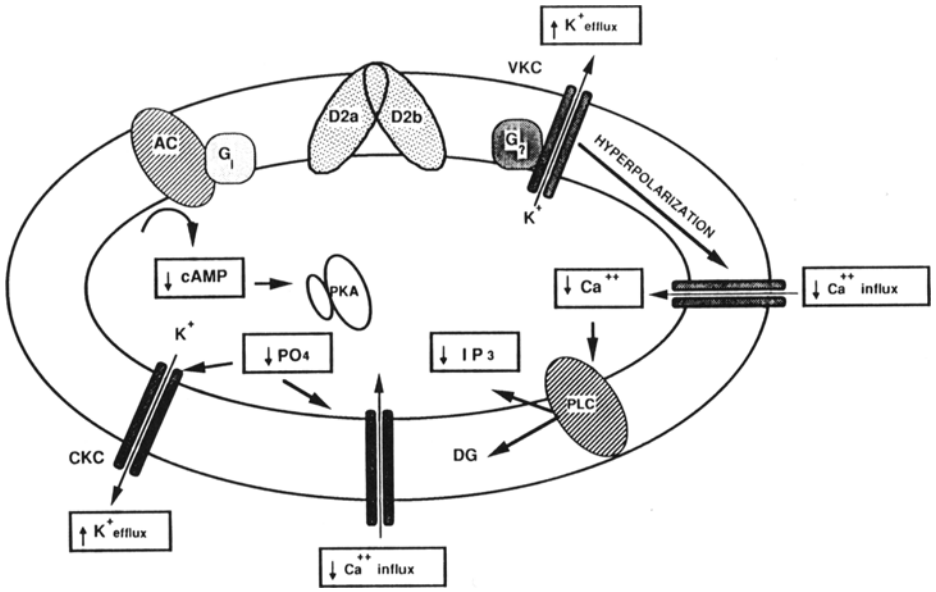


Fig. 1. Theoretical model of the supramolecular organization of dopamine D-2 receptors in cell membrane. Like other G-protein-coupled neurotransmitter receptors, the D-2-receptor multiprotein complex may include three basic components: the Detector (D-2), the coupling protein (G protein), and the effector (adenylyl cyclase [AC] or voltage-regulated K channels [VKC]). The diversity in the pharmacological and functional profiles of the different dopamine D-2 receptors in brain regions and peripheral tissues may stand on the cell-specific expression of those proteins involved in processing the dopamine signal.

Activation of each separate transduction pathway may induce a differential cascade of intracellular events and activate distinct enzymes or ion channels. In particular, the decrease in cAMP formation induced by D-2a stimulation may result in a series of intracellular events, including a reduction of the cAMP-dependent protein kinase (pKA) activity, a decrease in the phosphorylation state of proteins assembled in calcium channels (and/or K channels) to reduce the influx of calcium, and the permeability of the calcium-activated K channels (CKC).

On the other side, activation od D-2b may increase K permeability through voltage-dependent K channels (VKC) to hyperpolarize the cell membrane and decrease calcium influx. The reduction in the intracellular concentrations of ionized calcium may inhibit phospholipase C (PLC) activity and decrease inositolphosphate (IP) production.

ported by recent observations showing that the ratio between the mRNA levels for the different isoforms of the D-2 receptor can be modified by chronic treatment with antipsychotics.

## Conclusion

Receptor function is geared to detect specific chemical signals that reach the outer surface of the neurons, where the receptor is located, and to cause the internalization of these signals, transforming them into metabolic stimuli. Currently, dopamine D-2 receptors are considered to be important regulatory sites at both pre- and postsynaptic function; they adapt to environmental changes by virtue of their plasticity, which depends on a certain functional flexibility in their organization. The supramolecular complex of dopamine D-2 receptors resembles that of other G-protein-coupled neurotransmitter receptors, and it is believed to include three basic units: The detector or recognition site for dopamine; the coupler, which may be a G protein; and the effector.

Taking into account the intracellular modifications that follow dopamine D-2 receptor stimulation and the drug sensitivity of the different pathways, it can be proposed that diverse dopamine D-2 receptors are coupled to various amplifier systems with different molecular mechanims (Fig. 1).

Table 1
Comparison of the Amino Acid Sequences with D-2 and D-3 Receptors[a]

```
1
MDPLNLSWYDDDLERQ-NWSRPFNGSEGKADRPHYNYYAMLLTLLIFIIVFGNVLVCMAVSREKAL
* **         *              *     * ***  ***      **  * *** *** **  ** **
MAPLS-Q-ISTHLNSTCG-AENSTGVN-RA-RPH-AYYALSYCALILAIIFGNGLVCAAVLRERAL
1
66
QTTTNYLIVSLAVADLLVATLVMPWVVYLEVVGE-WKFSRIHCDIFVTLDVMMCTASILNLCAISI
******* ********************** *  * **** **  ********************
QTTTNYLVVSLAVADLLVATLVMPWVVYLEVTGGVWNFSRICCDVFVTLDVMMCTASILNLCAISI
61
131
DRYTAVAMPMLYNTRYS-SK-RRVTVMIAIVWVLSFTISCPLLFGLNNT-DQNECIIANPAFVVYS
****** **  *       * **  **  **** *  ******* * * *   * * ** **  **
DRYTAVVMPVHYQHGTGQSSCRRVALMITAVWVLAFAVSCPLLFGFNTTGDPSICSISNPDFVIYS
127
194
SIVSFYVPFIVTLLVYIKIYIVLRKR-RKRVNTKRSSR--AFRANLKTPLKGNC-T-HP-EDMKLC
* ******* ** ***  ****** * ***  *   *     *     *    *  **
SVVSFYVPFGVTVLVYARIYIVLRQRQRKRILTRQNSQCISIRPGF--PQQSSCLRLHPIRQFSIR
191
254
TVIMKSNGSFPVNR-RRMDAARRAQE-LEMEMLSSTSPPERTRYSPIPPSHHQLTLPDPS-HH-GL
                 * *    *    **   *                  *    * *
ARFLSDATG-QMEHIEDKQYPQKCQDPL-LSHLQPPSPGQ-TH-GGLKR-YYSIC-QDTALRHPSL
251
316
HSNPD-SPAKPEKNGHAKIVNPRIAKFFEIQTMPNGKTRTSLK-T-MSRRKLSQQKEKKATQMLAI
      **     *       *       *     **    ***       *      ******* *
EGGAGMSPVERTRNSLSPTMAPKLS-L-EVRKLSNGRLSTSLRLGPLQPRGVPL-REKKATQMVVI
314
379
VLGVFIICWLPFFITHILNIHCD-CNIPPVLYSAFTWLGYVNSAVNPIIYTTFNIEFRKAFMKILH
*** ** ****** ** ** ** *  *   * * * ********* **  ****** ******  ***
VLGAFIVCWLPFFLTHVLNTHCQACHVSPELYRATTWLGYVNSALNPVIYTTFNVEFRKAFLKILS
380
444
C    D-2 receptor
*
c    D-3 receptor
446
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[a]Putative transmembrane regions are boxed, * indicates amino acid conservation; the 29 amino acid sequence missing in the short isoform of D-2 receptor mRNA is underlined.

Pharmacologically, at least two distinct categories of dopamine D-2 receptors exist in rat pituitary. The first (D-2a) is insensitive to BHT 920 and coupled to inhibition of adenylyl cyclase activity; the second (D-2b) is activated by BHT 920 and linked to voltage-dependent K channels. The two types of dopamine D-2 receptors differ in their structure, G-protein-coupled, and effector. Each of the three basic receptor units shows a certain degree of heterogeneity, which may

affect the quality and the kinetic of the response. This variety may represent the molecular basis for the diversity in pharmacological and functional profiles of different dopamine D-2 receptors located in various brain areas and peripheral tissues.

Finally, it should be noted that the regulation of the dopamine-receptor gene expression is the focus of current interest for a better understanding of the antipsychotic mode of action. The specificity of antipsychotic medication could in fact be investigated by evaluating the change in the expression of the various molecular species of D-2 and D-3 receptors induced by either the disease or the drug treatment. This approach may open new vistas in the pharmacology of the dopamine receptors.

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