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# Coupling of $D_1$ and $D_5$ Dopamine Receptors to Multiple G Proteins

## *Implications for Understanding the Diversity in Receptor–G Protein Coupling*

***Anita Sidhu***

*Laboratory of Molecular Neurochemistry, Department of Pediatrics, Georgetown University Medical Center, Georgetown University, 3900 Reservoir Road NW, Washington, DC 20007*

## Abstract

Dopamine receptors are a subclass of the super family of G protein-coupled receptors, that transduce their effects by coupling to specific G proteins. Within the dopamine receptor family, the adenylyl cyclase stimulatory receptors include the $D_1$ and $D_5$ subtypes. The $D_1$ and $D_5$ dopamine receptors are genetically distinct, sharing >80% sequence homology within the highly conserved seven transmembrane spanning domains, but displaying only 50% overall homology at the amino acid level. When expressed in transfected $GH_4C_1$ rat pituitary cells, both $D_1$ and $D_5$ receptors stimulate adenylyl cyclase and have identical affinities toward dopaminergic agonists and antagonists. In order to analyze specific signaling pathways mediated by activation of either $D_1$ or $D_5$ receptors, we have identified the G proteins that are coupled to these receptors. Through functional analyses and competition binding studies, and from immunoprecipitation techniques, using antisera against the various $\alpha$ subunits of G proteins, we have established that both $D_1$ and $D_5$ receptors couple to $G_s\alpha$. In addition, $D_1$ receptors are also coupled to $G_o\alpha$. Since $G_o\alpha$ has been implicated in the regulation of $Ca^{2+}$, $K^+$, and $Na^+$ channels, this finding would suggest that $D_1$ receptors can mediate the functional activity of these ion channels. There is also evidence to indicate that $D_5$ receptors couple to $G_z\alpha$, a novel G protein abundantly expressed in neurons. Thus, despite similar pharmacological properties, such differential coupling of $D_1$ and $D_5$ receptors to G proteins other than $G_s\alpha$, indicates that dopamine can transduce varied signaling responses upon the simultaneous stimulation of both these receptors.

**Index Entries:** Dopamine receptors; G proteins; signal transduction.

## $D_1$ Family of Dopamine Receptors

Abnormal dopamine neurotransmission has been implicated in diverse neuropathological conditions, including schizophrenia, Parkinson's disease, hyperprolactemia, Tourette's syndrome, attention deficit disorder, and the reinforcing effects of drug and alcohol addic-

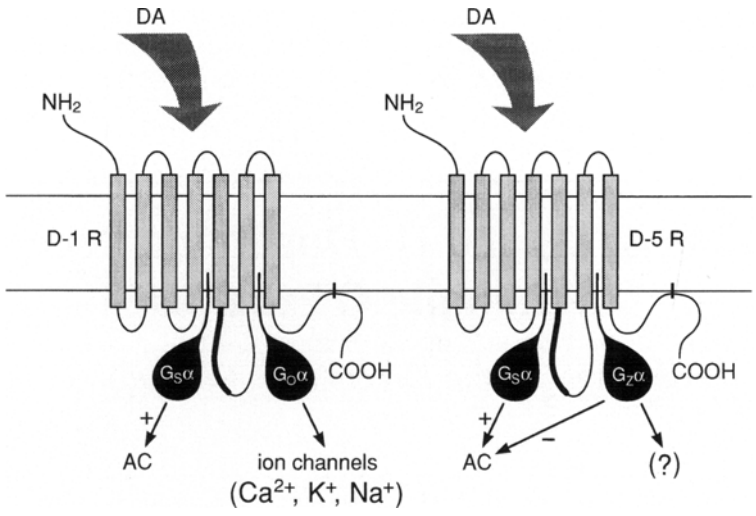


Fig. 1. Schematic representation of coupling of $D_1$ and $D_5$ receptors to different G proteins, through the third cytoplasmic loop of the receptors: The heavily drawn $NH_2$ terminus region of this loop, which shares 75% sequence homology, is depicted as the domain through which $D_1$ and $D_5$ sites interact with $G_s\alpha$. The coupling of the receptors to either $G_o\alpha$ or $G_z\alpha$, is shown to occur through the C-terminus portion of the third cytoplasmic loop. Abbreviations used are: AC, adenylyl cyclase; D-1 R, $D_1$ dopamine receptor; D-5 R, $D_5$ dopamine receptor; DA, dopamine.

tions. Dopamine exerts its effects in the central and peripheral nervous systems through at least seven genetically distinct receptor subtypes: $D_1$, $D_2$, $D_3$, $D_4$, $D_5$, $D_{1C}$, and $D_{1D}$ (Gingrich and Caron, 1993; O'Dowd, 1993; Sugamori et al., 1994; Demchyshyn et al., 1995). Each of these receptors is composed of seven α-helical transmembrane spanning regions linked by intracellular and extracellular loops of varying size (*see* Fig. 1). The dopamine-receptors transduce their signals by coupling to the heterotrimeric GTP binding proteins, termed G proteins, consisting of α, β and γ subunits (for reviews, *see* Hepler and Gilman, 1992; Exton, 1994; Neer, 1994). $D_2$-like dopamine receptors, $D_2$, $D_3$ and $D_4$, have introns in their coding region and exist in various forms by alternate splicing in the region of the third cytoplasmic loop. $D_2$ and $D_4$ receptors inhibit adenylyl cyclase by coupling to the inhibitory $G_i$ proteins (Gingrich and Caron, 1993; O'Dowd, 1993), whereas $D_3$ receptors only modulate cyclase function weakly.

Members of the $D_1$ family of receptors, $D_1$, $D_5$, $D_{1C}$, and $D_{1D}$, all stimulate adenylyl cyclase and are encoded by intronless genes, which show less diversity in their sequences than the $D_2$ receptor subtypes. $D_{1C}$ and $D_{1D}$ dopamine receptors are found in *Xenopus laevis* and avian systems, respectively, whereas the $D_1$ and $D_5$ dopamine receptors are predominantly found in mammalian species. In addition to their ability to stimulate cyclase, $D_1$ receptors have also been shown to mediate the functional activity of other signaling systems: stimulation of phospholipase C (Undie et al., 1994), inositol phosphate production and $Ca^{2+}$ mobilization in *Xenopus oocytes* (Mahan et al., 1990), inhibition of $Na^+/K^+$-ATPase (Bertorello et al., 1990) and the $Na^+/H^+$ antiporter (Felder et al., 1990), activation of the arachidonic acid cascade system (Piomelli et al., 1991) and stimulation of $K^+$ efflux (Laitinen, 1993).

Although $D_1$ and $D_5$ dopamine receptors appear to be virtually identical, the overall sequence homology between the two receptors

is only 50% (O'Dowd, 1993). $D_5$ receptors have been suggested to have higher affinity toward dopamine and lower affinity for the antagonist, (+)butaclamol, relative to $D_1$ (Grandy et al., 1991; Sunahara et al., 1991). However, we (Kimura et al., 1995a) and others (Hill et al., 1996) have not found any such pharmacological differences between $D_1$ and $D_5$ sites. More recently, we found that $D_5$ receptors cannot be covalently tagged by the $D_1$-selective photoaffinity ligand, [$^{125}$I]MAB, even though they bind the photolabel with identical affinity and stereospecificity as $D_1$ receptors (Sidhu et al., 1998a). This indicates that there are differences in amino acid sequences between the $D_1$ and $D_5$ receptors in close proximity to the ligand binding region, consistent with the low sequence homology of these proteins.

Because of the identical affinities of $D_1$ and $D_5$ receptors toward agonists and antagonists, and the lack of suitable pharmacological drugs that discriminate between these receptors, it is presently not possible to assign with certainty specific functions to the individual receptors. This problem is further compounded by studies in transfected cells that may or may not express the appropriate effector systems, regulatory components, and cofactors that are present in the native cellular environment of these receptors. Finally, in primate brains, there is considerable coexpression of $D_1$ and $D_5$ receptors in various brain regions, such as striatum, cerebral cortex, hippocampus, and in the lateral and medial thalamus (Bergson et al, 1995a; Bergson et al, 1995b; Choi et al., 1995). $D_1$ and $D_5$ coexpression has also been observed in peripheral tissues, such as renal cortex (Nash et al., 1993). It is possible that by stimulating adenylyl cyclase and perhaps transducing other, different signaling systems, cells that coexpress both $D_1$ and $D_5$ sites, cause an augmentation of dopamine effects, because of a redundancy phenomenon, while simultaneously eliciting dissimilar and hitherto unknown functions.

Given these limitations, we decided to focus our efforts in analyzing receptor-G protein coupling, hypothesizing that the identification of G proteins that couple to these receptors may enable predictions and initiate searches of specific signal-transducing pathways mediated by $D_1$ and $D_5$ receptor. Moreover, such studies would also be helpful in understanding how dopamine, and indeed other neurotransmitters, selectively and simultaneously activate receptors displaying similar affinities toward the agonist, when coexpressed within the same neuron. For example, $D_1$ and $D_2$ receptors, which are present in postsynaptic striatal neurons, stimulate and inhibit, respectively, adenylyl cyclase, which could, but does not, result in an overall negation of the dopaminergic signal. Furthermore, given the similar affinities of $D_1$ and $D_5$ sites for dopamine, how is a specific signaling pathway preferentially activated by these receptors? The key to such questions may lie in the nature of the G proteins that are coupled to these receptors.

## GTP-Binding Proteins

To date there are at least 21 distinct subtypes of the $\alpha$, 4 of the $\beta$ and 6 of the $\gamma$ subunits. The $\alpha$-subunit of G proteins, upon binding of GTP after activation by agonist-occupied receptor, mediates the activity of diverse signaling systems. For example, receptors stimulate adenylyl cyclase activity, resulting in synthesis of adenosine 3′,5′-monophosphate (cAMP), upon coupling of receptors to $G_s\alpha$, in response to agonists. In opposing pathways, receptors inhibit adenylyl cyclase by coupling to the inhibitory G proteins, such as $G_{i1}\alpha$, $G_{i2}\alpha$, or $G_{i3}\alpha$.

Although G protein-coupled receptors were initially believed to selectively activate a single effector, they are now known to have an intrinsic ability to generate multiple signals through interaction with different $\alpha$ subunits of G proteins (Birnbaumer et al., 1990; Milligan, 1993). Thus, the $\alpha_2$-adrenergic receptor is known to couple to all the pertussis toxin (PTX)-sensitive G proteins, $G_i$ ($G_{i1}$, $G_{i2}$, $G_{i3}$) (Kurose et al., 1991) and $G_o\alpha$ (Okuma and Reisine, 1992). $D_2$ dopamine receptors can inhibit adenylyl cyclase by

coupling to $G_{i2}\alpha$ and $G_{i3}\alpha$, but not $G_{i1}\alpha$ (O'Hara et al., 1996). The SSTR2 subtype of somatostatin receptors couples to $G_{i3}\alpha$ and $G_o\alpha$ (Law et al., 1991), whereas endothelin B receptors couple to both $G_s\alpha$ and $G_q\alpha$ (Jouneaux et al., 1994; Allgeier et al., 1994). The coupling of human parathyroid hormone receptor to $G_s$ and $G_i$ has also been demonstrated (Schneider et al., 1994). The ability of G protein-coupled receptors to couple to diverse α-subunits of G proteins is believed to occur primarily through the third cytoplasmic loop of the receptor (Fig. 1), a region that typically exhibits the largest sequence diversity among G protein-coupled receptors. Indeed, this sequence diversity may be key to the ability of receptors to couple to different G proteins.

## Coupling of $D_1$ Receptors to Different G Proteins

Through reconstitution studies, we initially demonstrated that $D_1$ dopamine receptors were also coupled to multiple G proteins (Sidhu et al., 1991). That $D_1$ receptors couple to $G_s\alpha$, is well established given its ability to stimulate adenylyl cyclase; $G_s\alpha$ remains the only Gα protein known to stimulate cyclase. To test if $D_1$ receptors were also coupled to other G proteins, soluble rat striatal $D_1$ receptors were reconstituted into phospholipid vesicles with exogenous G proteins from HeLa cells (Sidhu et al., 1991), after inactivation of endogenously present G proteins with *N*-ethylmaleimide, NEM, in the presence of a $D_1$ agonist, to protect the receptor (Sidhu et al., 1986). Agonist-competition binding studies were then performed using the $D_1$-selective antagonist, [$^{125}$I]SCH 23982 (Sidhu and Kebabian, 1985). The high affinity binding sites, which represents the activated, G protein-coupled state of the receptor, were measured.

Approximately 40–57% of the receptors were in the high-affinity state in the hybrid-reconstituted vesicles; these high-affinity sites were sensitive to modulation by Gpp(NH)p, the nonhydrolyzable analog of GTP, indicating coupling to the exogenous HeLa G proteins. If the HeLa cells were first treated with PTX, which inactivates $G_i\alpha$ and $G_o\alpha$ by ADP-ribosylation, the high affinity $D_1$-binding sites in the hybrid system were diminished by 50%, indicating that at least half of these hybrid high-affinity sites were caused by coupling of the receptor to PTX-sensitive G proteins. When $D_1$ receptors were reconstituted with G protein extracts from cyc$^-$ cells, which lack $G_s\alpha$, only 25% of the $D_1$ receptors continued to display Gpp(NH)p-sensitive high-affinity sites. Treatment of cyc$^-$ cells with PTX abolished all high-affinity sites, confirming coupling of the $D_1$ receptors to the PTX-sensitive G proteins, $G_i/G_o$ (Sidhu et al., 1991). These studies with the HeLa hybrid system indicated that not only did $D_1$ receptors couple to $G_i/G_o$, but that such coupling occurred in the simultaneous presence of $G_s\alpha$.

Although we documented the ability of $D_1$ sites to couple to multiple G proteins in reconstituted lipid vesicles, it was unclear if such coupling also existed in membranes, where lateral mobility between receptors and G proteins are likely to be restricted, limiting such interactions. In rat pituitary $GH_4C_1$ cells transfected with $D_1$ cDNA, 60% of the total membrane-bound $D_1$ receptor sites were in the high affinity state (Kimura et al., 1995a). If the $D_1$-$GH_4C_1$ cells were treated with either PTX or cholera toxin (CTX, which ADP-ribosylates $G_s\alpha$), the percentage of $D_1$ sites in the membranes was reduced to 45%. This suggests that even in the membrane-bound state, $D_1$ sites are able to couple to both CTX- and PTX-sensitive G proteins.

$D_1$ receptor–G protein coupling was also analyzed in human SK-N-MC neuroblastoma cells, which endogenously express these receptors (Sidhu and Fishman, 1990; Sidhu, 1997), and the results compared to those seen with $GH_4C_1$ cells (Kimura et al, 1995a). Unlike our findings in $GH_4C_1$ cells, in the SK-N-MC cells PTX-sensitive coupling was not observed (Kimura et al, 1995b). Using Gα-specific antisera (Lounsbury et al., 1993), we found that of the PTX-sensitive G proteins expressed in

$GH_4C_1$ cells, $G_{i2}\alpha$ and $G_o\alpha$ were not expressed in SK-N-MC cells, suggesting that the absence of PTX-sensitive $D_1$ coupling in SK-N-MC cells was caused by a lack of expression of these specific G proteins.

Using a novel immunoprecipitation approach employed previously for identifying G proteins coupled to $\alpha_2$-adrenergic and somatostatin receptors (Okuma and Reisine, 1992; Law et al., 1991), we confirmed the identity of the PTX-sensitive G protein coupling to $D_1$ sites to be $G_o\alpha$ (Kimura et al., 1995b). In this procedure, sodium cholate-solubilized $D_1$-$GH_4C_1$ cell membranes were incubated with various anti-$G\alpha$ antisera and the immune complex was precipitated by the addition of protein A Sepharose. Under these conditions, receptors coupled to G proteins remain associated with the $\alpha$-subunit and are coimmunoprecipitated with the $\alpha$-subunit. Through radioligand-binding studies with [$^{125}$I]SCH 23982, we tested for the presence of the $D_1$ receptor in the immunopellet, and documented a parallel loss of receptors from the soluble supernatant.

With anti-$G_o\alpha$ antiserum, 34% of the total soluble $D_1$ receptor binding activity was detected in immunopellets, indicating association between the receptor and $G_o\alpha$. Anti-$G_s\alpha$ antiserum coimmunoprecipitated 24% of the receptor-binding activity in the immunopellet. These coimmunoprecipitations of $D_1$-binding activity in the immunopellets were accompanied by a parallel loss in $D_1$ binding in the supernatant. When antisera directed against the alpha-subunits of $G_{i1}$, $G_{i2}$, $G_{i3}$, or $G_q$ were used, negligible $D_1$ binding was detected in the immunopellets (Kimura et al., 1995b).

That the $D_1$ dopamine receptor is able to couple to PTX-sensitive G proteins had been suggested earlier in rat renal tissues (Bertorello and Aperia, 1988). The inhibition of $Na^+/K^+$-ATPase activity seen in these studies required the simultaneous stimulation of both $D_1$ and $D_2$ dopamine receptors. Pretreatment of tissues with PTX abolished the $D_1/D_2$-mediated inhibition of $Na^+/K^+$-ATPase. However, these studies could not distinguish if the observed PTX-sensitivity was because of a G protein that was coupled to $D_2$ or to $D_1$ receptors.

The coupling of $D_1$ sites to $G_o$ predicts that these receptors modulate ion channels, since $G_o\alpha$ is known to couple to signal transducing systems that mediate ion channel function, such as $Ca^{2+}$, $K^+$, and $Na^+$ channels. Indeed, dopamine depresses and slows a voltage-dependent $Ca^{2+}$ current in embryonic chick sympathetic neuron, through a PTX-sensitive G protein (Hille, 1994). In *Xenopus* oocytes, $D_1$ receptors can induce $Ca^{2+}$ mobilization in a cAMP-independent manner (Mahan et al., 1990). In the resting state of medium-spiny neurons, $D_1$ dopamine receptors inactivate a slow $K^+$ current (Kitai and Surmeier, 1993). In all these studies, the $G\alpha$ mediating the dopamine response was not identified and it would appear that these effects probably occur through coupling of $D_1$ sites to $G_o\alpha$. Finally, coupling of $D_1$ receptors to PTX-sensitive G proteins causes suppression of adenylyl cyclase, in the absence of functional $D_1$ receptor/$G_s\alpha$ coupling (Kimura et al., 1995b). The ability of $D_1$ receptors to couple to $G_o\alpha$ will now enable confirmation of the involvement of this G protein in the $D_1$-mediated regulation of these diverse ion channels.

## Coupling of $D_5$ Dopamine Receptors to Different G Proteins

The ability of $D_5$ receptors to stimulate adenylyl cyclase, predicts that these receptors couple to $G_s\alpha$. However, $D_5$ receptors have also been shown to inhibit catecholamine secretion in bovine chromaffin cells (Dahmer and Senogles, 1996). Interestingly, in these cells, $D_5$ receptors were suggested not to be coupled to $G_s\alpha$, since only negligible dopamine-mediated activation of cyclase was observed. However, the $D_5$-mediated inhibition of catecholamine secretion was shown to occur through a PTX-insensitive G protein.

In transfected $GH_4C_1$ cells, we had earlier demonstrated that 48% of the membrane-bound $D_5$ receptors were in the high-affinity

state and could couple to both $G_s\alpha$ and to a PTX-insensitive G protein (Kimura et al., 1995a). In PTX-treated cells, approx 50% of the total receptor population continued to exist in the high-affinity state. Interestingly, we found that at least half of the $D_5$ high-affinity binding sites were much more resistant to modulation by Gpp(NH)p, when compared to the $D_1$ high-affinity sites (Kimura et al., 1995a). Indeed, high (300 $\mu M$) levels of Gpp(NH)p were necessary to abolish these sites, suggesting that this G protein had a slow rate of GTP/GDP exchange rate and/or a low intrinsic GTPase activity. However, in the presence of the G protein activator $AlF^-_4$ (20 $\mu M$ $AlCl_3$ and 10 m$M$ NaF), sensitivity to lower (100 $\mu M$) levels of Gpp(NH)p was appropriately restored. This indicated that the PTX-insensitive G protein coupling to $D_5$ receptors was either $G_q\alpha$ or $G_z\alpha$, both of which are resistant to PTX and require $AlF^-_4$ for activation.

We have recently shown that $D_5$ dopamine receptors couple to $G_z\alpha$, in addition to $G_s\alpha$ (Sidhu et al., 1997b). From immunoprecipitation studies with antisera against various $\alpha$-subunits of G proteins, we found that $D_5$ receptors were associated with $G_s\alpha$ and $G_z\alpha$. CTX treatment of transfected $D_5$-$GH_4C_1$ cells reduced, but did not abolish, the membrane-bound high-affinity binding sites. Additionally, anti-$G_s\alpha$ antiserum caused the coimmunoprecipitation of 33% of the soluble receptors. These studies indicate that $D_5$ receptors couple to $G_s\alpha$, in line with adenylyl cyclase stimulatory capability of these receptors.

Anti-$G_z\alpha$ antiserum coimmunoprecipitated 24% of the high-affinity binding sites, which corresponds to the $\%R_h$ of $D_5$ sites that are resistant to CTX (25%) and NEM (35%). The concurrent loss of $D_5$ receptor activity from the supernatant fraction when using anti-$G_z\alpha$ antiserum also supports our finding of association between the $D_5$ receptor and $G_z\alpha$. Despite similar pharmacological properties, $D_1$ receptors were not found to be associated with $G_z\alpha$ and no binding activity was detected in immunopellets with soluble D-1-$GH_4C_1$ membranes and $G_z\alpha$ antiserum under identical experimental conditions used for the D-5 studies. These results validate the specificity of $G_z\alpha$ and $D_5$ receptor associations.

That the $D_5$ receptors were coupled to $G_z\alpha$ was further confirmed by novel Gpp(NH)p release studies using protein A-conjugated iummunopellets obtained with $D_5$-$GH_4C_1$ extracts and anti-$G_z\alpha$ antiserum (Sidhu et al., 1998b). These immunopellets were treated with a $D_1/D_5$ agonist and increasing concentrations of Gpp(NH)p (up to 300 $\mu M$). Under these conditions, we predicted an uncoupling of the receptor from the protein A-conjugated G proteins, resulting in a Gpp(NH)p-dependent release of the $D_5$ receptors from the immunopellet. The released $D_5$ receptors were then detected by Western blots with anti-$D_5$-receptor antiserum. In the presence of agonist, but in the absence of Gpp(NH)p, there was little or no $D_5$ immunoreactivity released from the immunopellet. However, with increasing concentrations of Gpp(NH)p, there was a concomitant increase in the amount of receptor released from the immunopellet, confirming the $D_5/G_z\alpha$ association.

In other studies where antisera against both $G_s\alpha$ and $G_z\alpha$ were simultaneously used together in immunoprecipitation, the $D_5$ binding activity obtained in the immunopellets (57%) was additive to binding obtained in immunopellets when using either $G_s\alpha$ antiserum or $G_z\alpha$ antiserum alone. This value is also in agreement with the total receptor population existing in the high-affinity state (54%), indicating that associations between the $D_5$ receptors and $G_s\alpha/G_z\alpha$ occurs specifically through the high-affinity site of the receptor.

None of the other anti-G$\alpha$ antisera tested, including anti-$G_q\alpha$ antiserum, were able to coimmunoprecipitate appreciable levels of the $D_5$ receptor in the immunopellets (Sidhu et al., 1998b). We further eliminated the possibility that $D_5$ sites couple to $G_q\alpha$ based on the inability of $D_5$ receptors to mediate phosphatidylinositol mobilization upon activation of the receptors with dopamine. Moreover, treatment of $D_5$-$GH_4C_1$ membranes with NEM did not abolish the high-affinity binding sites. Since

NEM is known to inactivate both $G_s\alpha$ (Sidhu et al., 1986) and $G_q\alpha$ (Smrcka et al., 1991), NEM-treatment should have resulted in complete loss of the high-affinity sites, if the receptors were coupled to $G_q\alpha$. That $D_5$ receptors are not coupled to $G_o\alpha$ or any of the other PTX-sensitive G proteins, suggests that these receptors modulate signaling pathways that are distinct from those transduced by $D_1$.

## Functional Relevance of $D_5/G_z\alpha$ Coupling

Since the precise function of $G_z\alpha$ has not been definitively established, the molecular implications and relevance of $D_5/G_z\alpha$ coupling is not known. $G_z\alpha$ has been shown to mediate inhibition of adenylyl cyclase activity in certain cells (Wong et al., 1992; Kozasa and Gilman, 1995). In human embryo kidney 293 cells, dopamine, acting through the $D_2$ dopaminergic receptor inhibits hCG-stimulated cAMP accumulation and this inhibition occurred through coupling of receptor to $G_z\alpha$ (Wong et al., 1992). In $GH_4C_1$ cells, we did not find any $G_z\alpha$-mediated suppression of adenylyl cyclase in either CTX- or PTX-treated cells (Sidhu et al., 1997b) and it is not clear whether these cells express the necessary effector systems.

At least three types of adenylyl cyclases are present in $GH_4C_1$ cells: types II, IV, and VI, whereas type I adenylyl cyclase is not expressed (Paulssen et al., 1994); the expression of type V in these cells has not been reported to the best of our knowledge. Since $G_z\alpha$ inhibits type I and type V adenylyl cyclase activity (Kozasa and Gilman, 1995), the lack of expression of these cyclases in $GH_4C_1$ cells may account for the absence of cyclase inhibition mediated through $D_5/G_z\alpha$ coupling, even in CTX-treated cells.

Even though the signaling pathway(s) transduced through $D_5/G_z\alpha$ coupling is not clear, the functional relevance of such coupling becomes apparent upon examining brain regions that express this receptor. For instance, both type V adenylyl cyclase and $D_1$ dopamine receptors are expressed at high levels in the striatum, which has a rich dopaminergic input (Glatt and Snyder, 1993) and these receptors are known to stimulate the activity of this cyclase subtype (Yoshimura et al., 1996). By contrast, the hippocampus is rich in $D_5$, but not $D_1$, receptors, although type I adenylyl cyclase is abundantly expressed (Cooper et al., 1995). Therefore, in the striatum, which expresses lower levels of $D_5$ sites relative to $D_1$, activation of $D_1$ receptors by dopamine would lead to net activation, rather than inhibition, of adenylyl cyclase. In the hippocampus, however, the opposite may be true, where $D_5/G_z\alpha$ coupling is likely to predominate. Therefore, colocalization of $D_5$, $G_z\alpha$ and specific cyclase subtypes are likely to be of physiological relevance in striatum and hippocampus.

## Mechanism Through Which $D_1$ and $D_5$ Dopamine Receptors Can Simultaneously Couple to Multiple G Proteins

Although there is ample data indicating the ability of not only dopamine, but also other receptors, to couple simultaneously to diverse G proteins, the mechanism through which such coupling occurs is not known. However, a close examination of the structure of G protein-coupled receptors may provide certain clues (Fig. 1). These receptors interact with G proteins through the third intracellular loop of the receptor. The carboxyl terminus of the receptor is also thought to be important in such interactions, probably by facilitating the underlying process. Although the sizes of the third intracellular cytoplasmic loop is similar in $D_1$ and $D_5$ receptors, the sequence homology in this region is only 50%. Since both $D_1$ and $D_5$ receptors couple to $G_s\alpha$, such coupling must occur through a common site(s) localized on the third cytoplasmic loop. Sequence analysis of the third cytoplasmic loop of $D_1$ and $D_5$ receptors shows that the first 28 amino acids of the $NH_2$ terminus in this loop shares 75% se-

quence homology. We propose that the coupling of $D_1$ and $D_5$ sites to $G_s\alpha$ probably occurs through this stretch of amino acids of the $NH_2$ terminus. Indeed, an 11-amino acid $\beta_1$ adrenergic receptor substitution at the $NH_2$ terminus of the third intracellular loop of a chimeric $M_1$ muscarinic acetylcholine/$\beta$ adrenergic receptor is sufficient to allow the chimera to couple to $G_s\alpha$ (Wong et al., 1990).

The coupling of $D_1$ and $D_5$ receptors to diverse G proteins must then occur through the remaining amino acids of the third cytoplasmic loop that are nonhomologous, sharing only 42% sequence homology. Thus, alternative coupling of these receptors to different G proteins probably occurs through amino acids of this region, permitting the receptor to simultaneously couple to different G proteins, activating distinct signaling pathways within the cell, in response to stimulation by dopamine. A schematic drawing of this model is presented in Fig. 1. This model could also be applicable to other dopamine receptor subtypes coexpressed within the same neuronal cell. For example, dopamine could simultaneously stimulate both the $D_1$ and $D_2$ dopamine receptors, whose effects would be manifested through differential G protein coupling, such that the net result would not be a negation of the dopamine action (simultaneous stimulation and inhibition of adenylyl cyclase by $D_1$ and $D_2$ sites, respectively), but rather an augmentation of the net result.

There is also other indirect evidence supporting this model. For instance, all the $D_2$ and $D_4$ dopamine receptors exist as multiple isoforms because of splicing of the third cytoplasmic loop of the receptor gene (O'Dowd, 1993; Gingrich and Caron, 1993). Such splicing presents these receptor isoforms with multiple choices in receptor G protein coupling, while providing the necessary selectivity for the coupling.

## Conclusions

Although this review is focused on the $D_1$ and $D_5$ dopamine-receptor subtypes, the model proposed is applicable for understanding the mechanisms of action of other G protein-coupled receptor systems, which have virtually identical affinities for their own agonists and are coexpressed in the same cell. It is apparent that the diversity of G protein-coupled receptor subtypes, along with the heterotrimeric nature of the G proteins themselves, provides a complex signaling network within the neuronal cell. Several aspects of receptor-G protein coupling need to be resolved. These include deducing consensus sequences on the receptor for each $G\alpha$ subtype. It is also important to determine if other regions of the receptor, such as the carboxyl terminus, facilitate or actively participate in G protein coupling. Additionally, what is the mechanism by which a receptor can selectively discriminate between closely related subtypes of G proteins? For example, why does the $D_2$ dopamine receptor couple to the $\alpha$-subunits of $G_{i2}$ and $G_{i3}$, but not $G_{i1}$ (O'Hara et al., 1996)? It is likely that the specificity of such coupling is dictated by the receptor itself. As additional details become available in the future, the precise mechanism of receptor-G protein coupling will undoubtedly become known, providing a fascinating view of how signals are transduced, fine-tuned, and regulated within cells.

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