Specificity of Receptor-G Protein Interactions

DISCRIMINATION OF G; SUBTYPES BY THE D2 DOPAMINE RECEPTOR IN A RECONSTITUTED SYSTEM

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The selectivity of D2 dopamine receptor-guanine nucleotide-binding protein (G protein) coupling was studied by reconstitution techniques utilizing purified D2 dopamine receptors from bovine anterior pituitary and resolved G proteins from bovine brain, bovine pituitary, and human erythrocyte. Titration of a fixed receptor concentration with varying G protein concentrations revealed two aspects of receptor-G protein coupling. First, G12 appeared to couple selectively with the D2 receptor with ~10-fold higher affinity than any other tested Gi subtype. Second, the Gi proteins differed in the maximal receptor-mediated agonist stimulation of the intrinsic GTPase activity. G12 appeared to be maximally stimulated by agonist-receptor complex with turnover numbers of ~2 min⁻¹. The other Gi subtypes, G1, and G2, could be partially activated, resulting in maximal rates of GTPase of ~1 min⁻¹. Agonist-stimulated GTPase activity was not detected in preparations containing G1 from bovine brain. The differences in maximal agonist-stimulated GTPase rates observed among the Gi subtypes could be explained by differences in agonist-promoted guanyl nucleotide exchange. Both guanosine 5'-3-O-(thio)triphosphate (GTP·S) binding and GDP release parameters were enhanced 2-fold for the G4 subtype over the other Gi subtypes. These results suggest that even though several types of pertussis toxin substrate may exist in most tissues, a receptor may interact discretely with G proteins, thereby dictating signal transduction mechanisms.

In the pituitary, the major consequence of dopaminergic action is the inhibition of prolactin release from the anterior lobe. The action of dopamine in this tissue and other target tissues is presumably mediated through several transmembrane signaling pathways. The D2 dopamine receptor mediates inhibition of adenyl cyclase in striatum and the anterior and neurointermediate lobes of pituitary (Giannatsios et al., 1981; McDonald et al., 1984; Castelletti et al., 1989). A number of conflicting reports have suggested a role for this receptor in polyphosphoinositide metabolism (Canonicco et al., 1983, 1986; Simmons and Strange, 1985; Enjalbert et al., 1986; Journet et al., 1987) and Ca²⁺ influx (Enjalbert et al., 1988; Schofield, 1983; Login et al., 1988a, 1988b). More recently, the stimulation of this receptor has been shown to activate K⁺ channels in isolated primary lactotroph cells and striatal neurons (Castelletti et al., 1989; Margaroli et al., 1987; Vallar et al., 1988; Freedman and Weight, 1988). All of the observed effects of dopamine on various signaling systems are pertussis toxin sensitive, implicating the involvement of the G1/G4 family of proteins. At present, three forms of Gi which are distinct gene products have been identified by molecular biology techniques (Itie et al., 1986; Nakuda et al., 1986; Jones and Reed, 1987; Van Meurs et al., 1987; Michel et al., 1986; Bray et al., 1987). Several forms of Gi are present in virtually every cell or tissue studied to date. Thus, due to the multiplicity and ubiquity of these proteins, the question has arisen of whether the specificity of receptor-effector coupling resides at the receptor-G protein level, the G protein-effector, or both.

We have shown previously that partial purification of the pituitary D2 dopamine receptor by affinity chromatography results in co-purification of the receptor with a predominant pertussis toxin substrate of M, 40,000 (α subunit) (Senogles et al., 1987). These results suggested a certain selectivity between the interaction of the receptor and G proteins. In order to examine this question of specificity of signal transduction further, we reconstituted purified receptor and various resolved G proteins in phospholipid vesicles. The present studies reveal that the D2 dopamine receptor is able to discriminate G proteins in the G1/G4 family by two distinct mechanisms. The apparent affinity of the agonist-receptor complex for a given G protein is one such mechanism. The other is the ability of the receptor to stimulate (activate) a particular Gi subtype. Since these two mechanisms are evident in the interaction of G protein with the D2 dopamine receptor as well as rhodopsin, they may represent general mechanisms dictating the specificity of signal transduction.

EXPERIMENTAL PROCEDURES

Materials

[³²P]GTPyS, [γ-³²P]GTP, [³³P]GTP, and [³H]spiperone were obtained from Du Pont-New England Nuclear. App(NH)p and

1 The abbreviations used are: G1, G2, G4, G6, guanine nucleotide-binding proteins named according to the nomenclature proposed by Gilman (1987): SDS, sodium dodecyl sulfate; GTPyS, guanosine 5'-3-O-(thio)triphosphate; EGTA, [α,β-ethylenebis(oxyethyleneiminato)]tetraacetic acid; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; App(NH)p, adenylyl-5'-yl imidodiphosphate; Gpp(NH)p, guanylyl-5'-yl imidodiphosphate; DTT, dithiothreitol; BSA, bovine serum albumin.

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GTPyS were purchased from Boehringer Mannheim. Goat anti-rabbit IgG-conjugated alkaline phosphatase was obtained from Bio-Rad. Other chemicals and drugs were obtained from sources described previously (Senogles et al., 1986, 1987).

**Methods**

**Purification of D2 Dopamine Receptor from Anterior Pituitary**

The D2 receptor was purified as described below. Briefly, the solubilization and application to the affinity chromatography were carried out as described (Senogles et al., 1986) with the following changes. Once absorbed on the affinity chromatography matrix, receptor preparations were washed with 2 bed volumes of 100 µM Gpp(NH)p in the affinity chromatography wash buffer (0.1% digitonin, 50 mM Tris-HCl, pH 7.2, at 25 °C, 100 mM NaCl, 10 mM EDTA, 10 mM EGTA, 5 pg/ml each of leupeptin, pepstatin, aprotinin, and 100 µM phenylmethylsulfonyl fluoride). This wash step was necessary to remove the endogenously associated G protein that copurifies with the anterior pituitary receptor (Senogles et al., 1987). The receptor was eluted and further purified on Datura stramonium agglutinin lectin as described previously (Senogles et al., 1988). The specific activity of these preparations was routinely 2-4 nmol of [35S]GTPyS binding.

**Purification of Rhodopsin**

Rhodopsin was purified as described below. Briefly, the solubilization and application to the affinity chromatography were carried out as described (Senogles et al., 1986). The rhodopsin was eluted from the affinity column with a 300-ml gradient of 100-300 mM NaCl, 100 mM NaCl, 1 mM EDTA) to a final volume of 500 µl. G proteins (1-20 pmol) were added to the solution and incubated for 1 h on ice. The entire reconstitution was done under dark room conditions as much as possible, prior to assay. The samples were applied to an Extractigel columns as described above.

**Reconstitution of G Proteins and D2 Dopamine Receptor**

The co-reconstitution of receptor and G proteins was performed as follows. D2 dopamine receptor, usually 2-5 pmol (250 µl), in a buffer of 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM EDTA, 100 µM GTPyS, 0.1% ascorbate, 100 µM App(NH)p, 0.1% Lubrol, 1 µM GTPyS, 1 µM GDP (10,000 cpm/pmol), 2.5 mM MgCl2, 0.1 mM DTT, 0.1% ascorbate, 100 µM App(NH)p in a total volume of 0.1 ml. The bound ligand was separated from free by chromatography on Sephadex G-50 as described previously (Cerione et al., 1985). The agonist-sensitive [35S]GTPyS binding was performed on reconstituted vesicles using 50 mM Tris-HCl, pH 7.4, 100 mM NaCl, 100 µM App(NH)p, 0.5 mM EDTA, 100 µM GTPyS, 100 µM GDP, 0.1% Lubrol, 1 µM GTPyS, 1 µM GDP (10,000 cpm/pmol), 2.5 mM MgCl2, 0.1 mM DTT, 0.1% ascorbate. At zero time, 1 µL cold GTPyS along with either 10-6 M LY 171555 (D2-selective agonist) or haloperidol (antagonist) was added and the reaction terminated by filtration through BA 85 nitrocellulose membranes and washing with 2 X 5 ml of ice-cold 50 mM Tris-HCl, pH 7.2, 100 µM NaCl, 5 mM MgCl2. The filters were dried and dissolved in Lefkofluor (DuPont). The radioactivity was counted using a liquid scintillation counter.

**Data Analysis**

Turnover numbers for the various G proteins were determined as described by Brandt and Ross (1986). The CTPase and [35S]GTPyS binding were performed under identical conditions, as described above.

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and the molar turnover numbers were corrected for the agonist-sensitive G protein population. The first-order rate equation used to transform the $[^{35}S]GTP\gamma S$ and $[^{32}P]GDP$ release data is

$$(B_t - B/B_s) = e^{-kt}$$

The slope derived from linear regression analysis of $\ln (B_t - B/B_s)$ versus time yielded $K_{em}$, $B_t$ for the $[^{35}S]GTP\gamma S$ binding was the amount of $[^{35}S]GTP\gamma S$ bound after 1 h of incubation. For $[^{32}P]GDP$ release experiments, $B_t$ is the total $[^{32}P]GDP$ bound at zero time.

**Protein Determination**

Protein was quantitated by the method of Bradford (1976) using BSA as the protein standard.

**RESULTS**

**Identity and Purity of Various G Protein Preparations**

G proteins with α subunits of 41, 40, and 39 kDa were purified to apparent homogeneity from bovine brain. A 40-kDa G protein was purified from bovine anterior pituitary, and a 41-kDa G protein was purified from human erythrocyte. These resolved proteins were subjected to SDS-polyacrylamide gel electrophoresis, and the Coomassie Blue stain of this gel is shown in Fig. 1A. The identity and apparent purity of the G proteins were confirmed by Western blotting with G1 subtype-specific antipeptide antibodies characterized previously (Goldsmith et al. 1988a, 1988b) (Fig. 1B). The 41-kDa protein purified from brain was identified on the basis of reactivity with LD antisera to be $G_\alpha$. Both of the 40-kDa proteins from brain and pituitary were confirmed to be $G_{\alpha}$ on the basis of reactivity with LE3 antisera. The 41-kDa protein from human erythrocyte was identified as $G_{\alpha}$. The 39-kDa protein from brain was confirmed to be $G_\delta$ by cross-reactivity with GC antibody. The immunoblots were performed with two protein preparations: 500 ng (upper) and 5 μg (lower) for each G protein. The lower protein concentration is clearly detectable with our system and demonstrates that the preparations are >90% homogeneous with regard to subtype since no bands other than the major bands are detected at the higher protein concentration. The specific activity (data not shown) of these preparations (9–10 nmol of $[^{35}S]GTP\gamma S$ binding/mg of holoprotein) indicates that all of the proteins are >90% functional, as defined by their ability to bind GTP\γS (theoretical specific activity, ~11 nmol/mg for the holoprotein) and to serve as substrates for pertussis toxin (data not shown).

**Characterization of the $D_2$ Dopamine Receptor-G Protein Coupling in Phospholipid Vesicles**

The ability to detect agonist-dependent coupling between receptor and G protein depends greatly on the reconstitution and assay conditions. For example, addition of most exogenous detergents to promote micelle formation such as octyl β-D-glucoside, Lubrol PX, or cholate to the purified $D_2$ receptor from anterior pituitary prevented subsequent detection of agonist-dependent activation of the G protein after reconstitution. For this reason, the latter steps of the brain and pituitary G protein purification were carried out with CHAPS as the detergent, as CHAPS can be used in the reconstitution protocol without interference.

The agonist dependence of G protein activation was highly dependent on the free metal concentration. Fig. 2 shows the free [Mg$^{2+}$] dependence of both the basal (open circles) and agonist-stimulated (closed circles) $[^{35}S]GTP\gamma S$-binding activity of $G_{\alpha}$ reconstituted with $D_2$ receptor from bovine anterior pituitary. The $[^{35}S]GTP\gamma S$-binding activity of the basal condition displays a steep sigmoidal curve. The agonist-stimulated $[^{35}S]GTP\gamma S$-binding activity is shifted to the left of the basal condition and gives a plateau at ~1 mM free [Mg$^{2+}$]. The stimulation of $[^{35}S]GTP\gamma S$-binding activity by agonist diminishes at higher free [Mg$^{2+}$]. Indeed, >10 mM free [Mg$^{2+}$], the $[^{35}S]GTP\gamma S$-binding activity is no longer dependent on agonist. The maximum agonist stimulation was observed at ~1–2 mM free [Mg$^{2+}$], and 2 mM free [Mg$^{2+}$] was routinely used for assaying the reconstituted receptor and G proteins.

![Fig. 1. Analysis of purified G proteins by protein staining and immunoblotting](image1)

![Fig. 2. Effect of free [Mg$^{2+}$] on agonist-stimulated $[^{35}S]GTP\gamma S$ binding](image2)
Pattern of Agonist Dependence of the D₂ Dopamine Receptor-Gi₂ Interactions

To assess the relative affinities of various G proteins for the D₂ dopamine receptor, a fixed receptor concentration was reconstituted with increasing amounts of G proteins, and the G protein-receptor interactions were monitored by GTPase and [³⁵S]GTPyS binding. Fig. 3 shows the titration of D₂ dopamine receptor with Gi₂ isolated from bovine anterior pituitary. When G protein alone was reconstituted into phospholipid vesicles, GTPase activity for a fixed time was essentially linear with increasing concentrations of G protein in the vesicles (open circles). However, when the D₂ receptor (~75 fmol/reconstitution) was co-reconstituted with G protein and assayed in the presence (closed triangles) or absence (open triangles) of agonist, a deviation from linearity was observed. At low concentrations of Gi₂, GTPase activity in the presence of receptor was identical to that of the G protein alone. However, with increasing Gi₂ concentrations, the activity of the vesicles containing receptor and G protein was significantly higher than the vesicles with G protein alone. Thus, there appeared to be significant interaction of receptor and G protein at higher G protein/receptor ratios which was not agonist driven. These data were transformed to show the -fold stimulation by agonist of the GTPase turnover number at each of the G protein concentrations (Fig. 3, inset). Stimulation by agonist is biphasic over the range of G protein concentrations used in the experiments due to the lack of agonist-dependent activation at high G protein/receptor ratios. This pattern was consistently observed with all G₁ subtypes used in this study except G₅₂, which did not interact with the D₂ receptor (Fig. 4).

Specificity of G Protein-D₂ Dopamine Receptor Coupling Using G, Subtypes

The pituitary D₂ dopamine receptor was co-reconstituted with Gi₁, Gi₂, and Gi₃ from bovine brain; Gi₂ from bovine pituitary; and Gi₃ from human erythrocyte. The data were transformed as described for Fig. 3 and are shown in Fig. 4. The data obtained with Gi₂ from either the brain or pituitary source were indistinguishable in terms of this study. Reconstitution experiments using Gi₃ (closed squares) showed agonist activation at lower G protein/receptor ratios (maximal stimulation at ratios of 3–5) than any other G protein tested (maximal at G protein/receptor ratios of 25–30). Gi₂ from human erythrocyte (open triangles) and Gi₃ from brain (open circles) were maximally activated at approximately a 10-fold higher G protein/receptor ratio than Gi₅₂. The stimulation of the molar turnover number by agonist was ~10-fold with any of the G₁ subtypes. The addition of GDP to the incubation or reconstitution did not confer agonist stimulation to the Gi₂ preparations. However, these preparations of Gi₃ were tested with similar results. The addition of GDP to the incubation or reconstitution did not confer agonist stimulation to the Gi₂ preparations. However, these preparations of Gi₃ were active, as judged by stimulation by mastoparan (Higashijima et al., 1988), as ~10-fold stimulation of GTPase activity resulted with 100 µM mastoparan (data not shown).

Comparison of G Protein-Rhodopsin Coupling Using Transducin and G, Subtypes

To assess whether the pattern of coupling observed with the D₂ dopamine receptor was unique to this receptor, similar experiments were performed using rhodopsin purified from bovine rod outer segments. The data from titration of a fixed rhodopsin concentration with increasing amounts of various G proteins are shown in Fig. 5. Because of the inherent difficulty in obtaining truly inactive rhodopsin (absence of light), the data generated with vesicles containing the G protein alone were used as the basal rate. Since this parameter is linear with increasing G protein, the transformed data do not show the biphasic pattern observed with the D₂ receptor. Transducin is maximally stimulated by light-activated rhop-
Specificity of D₂ Dopamine Receptor Coupling to G Proteins

Dopamine at low G protein/rhodopsin ratios (G protein/receptor ratio of 2–3). G₁₁, G₁₂, and G₂ appear to be ~5–8-fold lower in affinity for rhodopsin compared with transducin, as their maximal activation occurs at G protein/receptor ratios of ~10. Rhodopsin was able to stimulate the turnover number for transducin ~10-fold, which is approximately 3-fold more than any of the other G₁ subtypes. Thus, the same patterns of specificity can be evidenced for rhodopsin/transducin as were obtained for the D₂ dopamine receptor/G₁₂.

Effect of Receptor-G Protein Coupling on Guanine Nucleotide Exchange

The functional differences in coupling with the D₂ dopamine receptor observed among G₁ subtypes in Fig. 4 were of two kinds: (a) affinity of the receptor for the G protein; and (b) ability of the agonist-receptor complex to stimulate the intrinsic GTPase activity. The GTPase cycle can be thought of as two kinetic processes, as reviewed recently by Freissmuth et al. (1989). The basal GTPase activity is influenced by the rates of the hydrolytic step of GTP cleavage and the rate of release of the product, GDP. Much evidence (reviewed by Gilman, 1987) has suggested that the rate-limiting step for basal GTPase in the cycle is the release of GDP, which is 1/10 as fast as the hydrolytic step. We chose to investigate the guanine nucleotide exchange reactions in order to explain the

**TABLE I**

Comparison of [³²S]GTP γS binding and [³²P]GDP release promoted by D₂ dopamine receptor

| G protein | [³²S]GTP γS binding | [³²P]GDP release |
|-----------|---------------------|------------------|
|           | Kᵦ (min⁻¹) | n | Kᵦ (min⁻¹) | n |
| G₁₁       | 0.08 ± 0.03 | 3 | 0.16 ± 0.02 | 4 |
| + Agonist | 0.24 ± 0.07 | 3 | 0.33 ± 0.08 | 4 |
| G₁₂       | 0.10 ± 0.03 | 3 | 0.16 ± 0.01 | 4 |
| + Agonist | 0.57 ± 0.10 | 3 | 0.78 ± 0.13 | 5 |
| G₁₃       | 0.11 ± 0.01 | 3 | 0.15 ± 0.01 | 3 |
| + Agonist | 0.28 ± 0.06 | 3 | 0.40 ± 0.11 | 3 |
| G₂        | 0.08 ± 0.02 | 4 | 0.18 ± 0.02 | 5 |
| + Agonist | 0.09 ± 0.02 | 4 | 0.17 ± 0.03 | 5 |

The symbols represent: transducin (×), G₁₁ (○), G₁₂ (■), G₁₃ (△), and G₂ (▼). The basal GTPase rates for these preparations were 0.16–0.19 mol of Pi released/min/mol of G protein.

**FIG. 6.** Comparison of [³²S]GTP γS binding promoted by the D₂ dopamine receptor. The D₂ dopamine receptor (1 pmol) was co-reconstituted with ~5 pmol of G₁₁ or ~20 pmol of G₁₂, G₁₃, or G₂. Agonist (filled symbols) or buffer control (open symbols) was added at time zero, and at various time points the assay was terminated by filtration. The symbols represent: G₁₁ (○), G₁₂ (□), G₁₃ (△), and G₂ (▼). B, represents the total amount of [³²S]GTP γS binding at 1 h.
**DISCUSSION**

Previous studies of receptor G protein coupling have revealed some striking differences in the patterns of coupling. For example, the β-adrenergic receptor couples to G, more efficiently than either G, Gm, or transducin (Cerion et al., 1985), as evidenced by agonist stimulation of GTPase activity. By the same criteria, the αβ-adrenergic receptor appears to couple more efficiently with either G, ~ G, > transducin > G, (Cerione et al., 1986). However, studies that have attempted to delineate receptor-G protein coupling further have failed to show receptor-mediated specificity within the Gi or Gg isoforms. Reconstitution studies with crude D2 dopamine receptors (O’Hara et al., 1988), muscarinic receptors (Haga et al., 1985, 1986; Kurose et al., 1986), or partially purified prostaglandin E receptors (Negishi et al., 1988) have shown no detectable differences in receptor-G protein coupling with G, or any Gg. Studies exploring G protein-effector coupling have also failed to show selectivity. Recent work with the four isoforms of G, have revealed no detectable differences in coupling directly to Ca2+ channel or adenylyl cyclase (Mattera et al., 1989). Three forms of G (G, G2, G3, G4) have been shown to activate the atrial K+ channel with approximately equal potency (Yatani et al., 1988). These studies suggest that G proteins may be multifunctional in terms of receptors and effectors. However, in the cell, some level of discrimination is required since receptors do not randomly activate all signal transduction pathways.

The experiments reported here utilized a reconstitution system, using the purified D2 dopamine receptor from bovine anterior pituitary and G proteins purified from several sources, to examine questions of receptor-G protein coupling. This paper documents several aspects of receptor-G protein coupling which may give insights into selectivity and specificity of these interactions. Two aspects of G protein-receptor interactions appeared to distinguish the various preparations of G proteins. There was a striking difference in apparent affinity between the receptor and the various subtypes of the inhibitory G proteins, as well as a difference in the ability of the agonist-receptor complex to stimulate the intrinsic GTPase activity of the G protein (efficacy).

The D2 dopamine receptor appeared to couple with ~10-fold higher affinity to G, as evidenced by lower G protein/receptor ratios for achieving maximum coupling. The source of G, was not critical, as brain and pituitary proteins were indistinguishable in all of the functional assays. These data indicating G2 couples with apparently higher affinity are in agreement with previous studies from this laboratory (Senges et al., 1987). The experiments utilizing rhodopsin also supported the observation of apparent differences in affinity for receptor-G protein coupling. Transducin clearly couples with higher affinity to rhodopsin than other G protein preparations, and rhodopsin varies in its ability to stimulate the GTPase activity of these proteins. This agrees with previous observations by Cerione et al. (1985).

The D2 dopamine receptor also appeared to activate the various G subtypes to different extents, as evidenced by stimulation of the intrinsic GTPase. Agonist-stimulated GTPase activity obtained with Gg yielded a turnover number of ~2 mol/min/mol of G protein, which is close to the upper catalytic limit (Gilman, 1987). However, G, and Gm were only able to achieve rates of ~0.8-1.0 mol/min/mol of G protein under agonist-stimulated conditions.

The maximal rates of GTPase under high [Mg2+] conditions were similar for all of the G protein preparations. This indicates that the differences observed in agonist-stimulated turnover numbers were not due to large intrinsic differences in

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**Fig. 7. Comparison of [α-32P]GDP release promoted by the D2 dopamine receptor.** The same concentrations of D2 dopamine receptor and G protein were used as in Fig. 6. The loading of the G proteins with [α-32P]GTP was performed as described under “Methods.” At time zero, agonist + 1 μM GTPγS was added. At various time points, the assay was terminated by filtration. B was the total amount of [32P]GDP bound at time zero. The symbols represent: basal (□), G (○), G, (■), G, (△), G, (▲).
GTPase activity but reflected a difference in receptor-G protein coupling. Interestingly, brain Gβs displayed no detectable interaction with the D₁ dopamine receptor. Several preparations of Gαs from brain were used and documented to be active either by coupling to rhodopsin or activation by mastoparan. In our previous studies (Senogles et al., 1987), we documented that immunoreactive Gαα proteins co-purified with the D₂ dopamine receptor from pituitary. A minor component of the total Gα protein was recognized by a Gαα-specific antibody, whereas the major component reacted with an antibody that recognizes Gβ subtypes. This observation would seem to be at odds with our observation that brain Gβs do not functionally couple with the D₁ dopamine receptor from pituitary. However, several forms of Gαα have been documented (Goldsmith et al., 1988a), and the Gαα species purified from brain and used in these studies may not correspond to the species detected in pituitary.

The observed differences in maximal catalytic rate attained by Gαs could be accounted for by enhanced guanine nucleotide exchange under conditions of agonist stimulation. Both the rate of GTP binding (as inferred by the rate of [35S]GTP–S binding) and GDP release from the Gαs were stimulated by D₂ dopamine receptor in the presence of agonist.

Reconstitution procedures utilizing receptor and G proteins purified by conventional chromatography have one major caveat: the purity of the various components. We have documented by protein staining and Western blotting the integrity of our G protein preparations. The differences observed in the reconstitution experiments, i.e., apparent differences in affinity or activation of G proteins, could not be accounted for by a low potential level of contamination (Fig. 1B). The results presented here have demonstrated that various forms of Gαs are not equivalent with regard to receptor interactions and that specificity can be demonstrated using a reconstituted system. The significance of these findings, in terms of determining the specificity of signal transduction for a given receptor, remains to be explored further.

The D₁ dopamine receptor in anterior pituitary signals through at least two distinct pathways. The D₁ receptor mediates inhibition of adenyl cyclase, but this pathway does not account for all of the effects of dopamine on prolactin secretion. Dopamine, even in the presence of elevated cAMP, still mediates inhibition of prolactin release, indicating the existence of a cAMP-independent mechanism (Tam and D’Anjies, 1981). The D₂ receptor has also been shown to cause activation of K⁺ channels, and this effect through the K⁺ channel may in turn regulate voltage-sensitive Ca²⁺ channels. These pathways may account for the cAMP-independent effects of dopamine on prolactin secretion (Margaroli et al., 1987). A question of interest is whether these effectors couple to the D₂ receptor through one distinct type of G protein or a network of G proteins. Using patch-clamping techniques, no specificity has been observed among the native or recombinant Gα family for direct activation of the chick atrial K⁺ channel (Yatani et al., 1988). These data suggest that the specificity of coupling is not present at the level of G protein effector or either too subtle to be observed in the experimental design used.

The receptor-G protein-coupling properties documented here appear to have two distinguishing characteristics that could account at least in part for biological specificity: the apparent affinity of the receptor-G protein complex, and the ability of the receptor-agonist complex to activate the intrinsic GTPase. In terms of signal transduction, a logical consequence of D₁ dopamine receptor activation may be a preferential activation of Gαα by one or both mechanisms. The apparent differences in affinity between Gα₁ and the other Gα subtypes in coupling with the D₁ dopamine receptor are of unknown significance since the ratios of Gα proteins vary greatly with tissue source. Thus, in some tissues, this aspect of affinity may solely dictate coupling specificity. The ability of the receptor to activate Gα proteins to varying degrees is another mechanism for dictating specificity of coupling. For example, in the system described, the Gα₁ protein is activated maximally by D₁ dopamine receptor. The amount of the activated subunit, and not the specific subtype, released by receptor activation may influence the choice of effector system. This specificity could be afforded by the existence in the membrane of a loosely associated multifunctional complex made up of receptor-G protein-effector similar to the many multi-enzyme complexes documented (Sere, 1987). It is possible that the true biochemical mechanisms underlying receptor-effector coupling may involve both of these characteristics. Other biochemical or architectural events may also influence the receptor-G protein-effector coupling specificity and allow for a preferential activation of selective signaling pathways. Ultimately, the question of receptor-G protein-effector specificity will have to be examined using molecular biology techniques. Constructing a cell that expresses receptor, effector, and a single functional G protein may ultimately provide the necessary insights into the levels of control which must exist to account for the specificity of receptor signaling.

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