High Agonist-independent Activity Is a Distinguishing Feature of the Dopamine D1B Receptor Subtype*

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Dopamine D1A and D1B receptor subtypes belong to the superfamily of G protein-coupled receptors. Both receptors are coupled to the activation of adenylyl cyclase and exhibit distinct brain distribution. To identify functional differences, binding and stimulation of adenylyl cyclase were assessed in 293 cells expressing transiently either dopamine D1A or D1B receptors. Membranes expressing D1B receptors displayed higher affinities for agonists than those expressing D1A receptors, whereas antagonist affinities were lower at the D1B than at the D1A receptor. Basal activity of adenylyl cyclase in whole 293 cells expressing various levels of D1B receptors was significantly higher than the basal activity measured in cells expressing D1A receptors. Maximal activation of adenylyl cyclase resulting from stimulation of the D1B receptor was less than that obtained following agonist activation of the D1A receptor. In cells expressing D1B receptors, agonists displayed an increased potency for stimulating adenylyl cyclase in comparison with the potencies determined for the D1A receptor. On the other hand, certain antagonists displayed "negative efficacy" at both receptor subtypes but had a more profound inhibition on the agonist-independent signaling activity of the D1B receptor. The properties described here are reminiscent of those of constitutively active G protein-coupled receptors obtained by site-directed mutations. Thus, the D1B receptor may represent a naturally occurring receptor subtype with properties akin to those of constitutively active G protein-coupled receptors. The different anatomical distribution and biochemical properties of these D1 receptors strengthen the functional distinctions between the two subtypes and could account for the basis of heterogeneity within a given class of neurotransmitter or hormone receptors. In addition, if these properties are recapitulated in cells expressing the D1B receptors, they may underlie important role in the regulation of physiological effects by dopamine. Finally, these results raise the interesting possibility that psychotropic antagonist drugs used in the management of certain brain disorders may have their beneficial actions as negative efficacy compounds.

The recent cloning of several genes encoding different G protein-coupled dopamine receptors has provided a glimpse of the complexity underlying dopaminergic neurotransmission in the central nervous system. Five distinct genes encoding dopamine receptors have been isolated and characterized so far (1, 2). Among these, two genes encode dopamine D1-like receptors and are referred to as D1A and D1B subtypes (also referred to as D1 and D5, respectively). Both of these receptor subtypes have been shown to couple to activation of adenylyl cyclase (1, 2). Expression in COS-7 cells reveals that dopamine has higher affinity for the D1B dopamine receptor in comparison with the affinity of dopamine for the D1A receptor. Moreover, we have reported a differential mRNA distribution of these two D1 receptor subtypes in the rat brain (3). The classical D1A receptor is mainly found in the basal ganglia and more specifically in the nucleus accumbens, striatum, and olfactory tubercles. In marked contrast, the mRNA for the D1B receptor is virtually absent in the basal ganglia but is associated with limbic areas (3). Indeed, the D1B mRNA has been shown to be localized in the hippocampus, mammillary nuclei, and the parafascicular nucleus of the thalamus in rat brain (3, 4).

The functional and biochemical consequences of D1 receptor heterogeneity are difficult to assess since no subtype-selective agonists or antagonists yet exist. Despite the fact that these receptor subtypes show a high degree of sequence identity (~80% within their putative transmembrane regions), their extracellular and intracellular domains diverge most noticeably. Differences in the intracellular domains may suggest differences in the coupling and/or regulatory properties of these receptors. Whereas interesting differences have been observed in the regulatory processes within a given receptor subfamily (e.g., β1, β2, and β3 receptors) (5, 6), relatively few differences have been found when examining the intrinsic coupling properties of G protein-coupled receptors (7-12). These studies have provided insights in the role played by these cytoplasmic domains as important structural determinants involved in the determination of the binding and coupling properties of G protein-coupled receptors. Indeed, site-directed mutagenesis performed in the carboxyb-terminal region of the third cytoplasmic loop of the G protein-coupled adrenergic receptor (AR) resulted in constitutively active mutant receptors, i.e., activated receptors in the absence of agonist (7, 8). Similar mutations have led to the constitutive activation of β2-AR (G protein-coupled receptor) and α2-AR (G protein-coupled receptor) (9, 10). Moreover, substitution and deletion mutations in the third cytoplasmic loop of the yeast a-factor receptor produce a constitutive and hypersensitive phenotype (13). Overall, mutagenesis studies performed on...
adrenergic receptors have suggested that the third cytoplasmic loop plays a role in constraining the receptor into a mostly inactive conformation in the absence of agonist. These studies have led to the notion that an isomerization step must regulate formation of the receptor conformation capable of high affinity interaction with the G protein ([R•R*] (9, 12). The R* state is believed to be the receptor conformation capable of interacting with the G protein. Therefore, constitutive activity of G protein-coupled receptors would be explained by a shift of the conformational equilibrium to the active state of the receptor (R*).

Moreover, computer simulations and experimental data obtained with these constitutively active mutant receptors have helped to define several general properties these receptors have in common: (i) generation of a signal in the absence of agonist occupancy; (ii) increased agonist affinity for binding to the receptor; (iii) increased potency of agonists for stimulation or inhibition of the effector response; and (iv) increased intrinsic activity of partial agonists. Moreover, using the high basal activity of these mutant receptors, studies have shown that some classical antagonists display "negative efficacy" properties, i.e., antagonists can reverse the basal activity of receptors (14).

In the light of the initial observation that D1B receptors showed a higher binding affinity than the D1A receptor for dopamine (3, 15-17), as well as consistent findings of a higher basal activity in membranes prepared from a mouse Ltk- clonal cell line expressing the rat D1B receptor subtype (3), we have looked in more detail at the binding and coupling properties of the D1 receptor subtypes transiently expressed in human embryonic kidney cells (293 cells). This cell system has been widely used to characterize signaling pathways of different receptors in a transient expression assay (18-20). We report here that when studied in direct comparison with the D1A receptor subtype, the D1B receptor possesses properties similar to those of constitutively active mutant G protein-coupled receptors. Thus, the dopamine D1B receptor may represent a naturally occurring receptor subtype that shares biochemical properties with constitutively active mutant G protein-coupled receptors.

EXPERIMENTAL PROCEDURES

Plasmid Construction—A full-length genomic clone encoding the rat dopamine D1A receptor gene was isolated from a rat genomic library as described previously (3). Using the polymerase chain reaction, restriction sites were engineered at the 5' and 3' ends of the coding sequences of the rat D1A (SalI and BamHI) and rat D1B (HindIII and XbaI) genomic clones and subcloned into the expression vector pCMV5 (21). Sequence analysis was performed by dideoxy sequencing of double-stranded DNA using Sequenase (U.S. Biochemical Corp.). Expression constructs for human D1A and D1B receptors were in pCMV5 as described previously (22, 23).

Cell Culture and Plasmid Transfection—Human embryonic kidney cells (293 cells) were grown in minimal essential medium with Earle's salts, supplemented with heat-inactivated fetal bovine serum (10% v/v) and gentamicin (100 μg/ml). Cells were seeded in 100-mm dishes (~2 × 10^6 cells/dish) and transiently transfected using a modified calcium phosphate method (24). For binding studies, the amount of DNA was 8–10 μg/dish for maximal expression. For cAMP studies, the amount of DNA ranged from 0.001 to 10 μg/dish to obtain a spectrum of expression levels as reported previously (19). The total amount of DNA was kept constant at 10 μg/dish using wild type pCMV5 vector. All experiments were done with cells passaged 34–48 times. Using a 12CA5 epitope-tagged D1A and D1B we have been able to establish by flow cytometry that transfection efficiency is generally about 80–90%.

Ligand Binding—Following transfection (18–24 h), 293 cells were shocked with phosphate-buffered saline containing 15% glycerol (30–60 s), washed once with phosphate-buffered saline only, and fed with fresh culture medium. After a recovery period (4–6 h), cells were resuspended in 150-mm dishes and allowed to grow for an additional 32–48 h. To prepare membranes, cells were first washed once with cold phosphate-buffered saline and lysed in ice-cold buffer containing 10 mM Tris-HCl, 5 mM EDTA at pH 7.4 and centrifuged at 40,000 × g for 30 min. At the end of the centrifugation, pellets were resuspended in lysis buffer using a Brinkmann Polytron (18,000 rpm for 10–15 s) and stored at −80 °C until used. Frozen membranes were thawed and resuspended in binding buffer (50 mM Tris-HCl, 120 mM NaCl, 5.0 mM KCl, 4.0 mM MgCl₂, 1.6 mM CaCl₂, and 1.0 mM EDTA, pH 7.4, at 25 °C). Receptor binding was measured using [3H]SCH 23982 which was prepared by radioiodination of the deschloro analog of SCH 23390. Saturation binding studies were performed with increasing concentrations of [3H]SCH 23982 (0.05–5 nM). Competition binding studies were done using a constant concentration of [3H]SCH 23982 (~0.7 nM) and increasing concentrations of cold agonists or antagonists. Binding assays with agonists were performed in the presence of a final concentration of 0.1 mM ascorbate. Nonspecific binding was determined in the presence of 10 μM flupentixol. Binding assays were incubated for 90 min at 25 °C and were stopped on glass fiber filters (Whatman, GF/C) by vacuum filtration. The filters were washed four times with 4 ml of cold wash buffer (50 mM Tris, 120 mM NaCl, pH 7.2) and counted in a γ-counter. Protein concentration was determined using the Bio-Rad assay kit with bovine serum albumin as the standard.

cAMP Formation Assay—Twenty-four h after transfection, cells were seeded in six-well dishes. The next day, culture medium was replaced with fresh minimal essential medium containing fetal bovine serum (5% v/v), gentamicin, and [3H]adenine (1.5–2.0 μCi/ml). Cells were labeled for 18–22 h. Determinations of intracellular cAMP were done by incubating the cells in 10 μM HEPES-buffered minimal essential medium (40 mM HEPES, 1.0 mM MgSO₄, 2.5 mM KCl, 1.0 mM NaHCO₃, 0.25 mM Na₂HPO₄, 0.1 mM L-glutamine, 10% dialyzed fetal bovine serum, 50 μM ascorbate, and 1 mM L-methionine) for 30 min (37 °C) or as indicated in the text. Assays were terminated by aspiration of medium, addition of 1 ml of stop solution (2.5% v/v perchloric acid, 100 μM cAMP, and ~10,000 cpm of [3H]cAMP) and were placed in the cold for 20–30 min. The acid-cell lysates were transferred to tubes containing 0.1 ml of 4.2 KCl, and the salt precipitates were pelleted by centrifugation. Separation of [3H]cAMP in the cell extracts was performed using a sequential chromatography on Dowex and alumina columns as described previously (25). Data are presented as 1,000 times the ratio of [3H]cAMP formed over the total uptake measured in the well.

RESULTS

Membranes prepared from transiently transfected 293 cells expressing high levels of D1A and D1B receptors (~2 pmol/mg protein) were used to characterize these receptors by ligand binding. Saturation binding studies performed in 293 membranes expressing either the human D1A or D1B receptors revealed that [3H]SCH 23982 binds to a homogeneous class of receptors with a KD of 0.83 ± 0.10 nM (n = 5) and 0.84 ± 0.17 nM (n = 6), respectively (geometric mean ± S.E.). At the human D1B receptor, the agonists dopamine, (~)-apomorphine, and fenoldopam displayed a higher binding affinity than at the human D1A receptor (Table I). However, SKF 38393 had a similar affinity at both receptor subtypes. In contrast, all antagonists tested in the present study had a lower binding affinity for the human D1B receptor than for the human D1A receptor (Table I). These results are in agreement with the pharmacological profile reported previously in COS-7 cells (3, 23).

3 M. Tiberi and M. G. Caron, unpublished data.
4 L. S. Barak, M. Tiberi, and M. G. Caron, unpublished observation.
Human dopamine D1A and D1B receptors were expressed transiently using 5–10 μg of DNA/2.5 x 10⁶ cells. Competition binding curves were performed as described under "Experimental Procedures." Results are expressed as geometric means ± S.E. of the number of independent experiments as indicated in parentheses. All curves displayed a slope factor near unity and were fitted best to a one-site model using LIGAND (27).

**TABLE I**

<table>
<thead>
<tr>
<th>Agonists</th>
<th>Human D1A KD</th>
<th>Human D1B KD</th>
<th>Ratio D1B/D1A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenoldopam (n = 5)</td>
<td>28 ± 2</td>
<td>16 ± 1</td>
<td>0.6</td>
</tr>
<tr>
<td>SKF 38393 (n = 3)</td>
<td>61 ± 1</td>
<td>69 ± 1</td>
<td>1.1</td>
</tr>
<tr>
<td>(-)-Apomorphine (n = 5)</td>
<td>456 ± 45</td>
<td>328 ± 43</td>
<td>0.7</td>
</tr>
<tr>
<td>Dopamine (n = 5)</td>
<td>9,400 ± 282</td>
<td>1,080 ± 36</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Antagonists

| SCH 23391 (n = 6) | 0.23 ± 0.01 | 0.50 ± 0.01 | 2.2 |
| (+)-Butaclamol (n = 4) | 4.6 ± 0.17 | 3.2 ± 2 | 7.0 |
| Flupentixol (n = 5) | 4.6 ± 0.65 | 10 ± 0.8 | 2.2 |

The first manifestation that the D1B receptor might have properties reminiscent of constitutively active mutant receptors was a consistent elevation of the basal activity of adenylyl cyclase. As shown in Fig. 1 at equivalent expression levels, cells expressing either human or rat D1B receptors display a basal activity which is 2–4-fold higher than the basal activity obtained with either the rat or human D1A receptor. Stimulation of the rat or human D1A receptor with 10 μM dopamine for 30 min leads to a maximal activation greater than the one measured for the human or rat D1B receptor under the same experimental conditions. It is worth noting that the formation of cAMP upon exposure to dopamine was linear up to 45 min for both receptor subtypes (data not shown). To rule out any effect of the serum during [³H]adenine labeling of the cells, experiments were performed in which cells were labeled in serum-free media or in media containing a growth supplement (insulin/transferrin/selenite). The basal activities of the human D1A and D1B receptors were unaltered by these different labeling conditions (data not shown). These results thus provide the first evidence that the D1B receptor might have a significant agonist-independent activity in comparison with the D1A receptor. Importantly, this increased ability to activate the G protein in the absence of agonist was observed for both the human and rat homologue of D1B receptor.

To test further the hypothesis that the high agonist-independent activity of D1B receptor is not associated simply with high level of receptor expression, we examined the relationship among the receptor density, basal activity, and dopamine-stimulated adenylyl cyclase in 293 cells expressing transiently either the D1A or D1B dopamine receptors. Various levels of expression of dopamine receptor subtypes were obtained in 293 cells by increasing the quantity of DNA in the transfection procedure (19). As shown in Fig. 2, the basal level of intracellular cAMP increases linearly as a function of increasing receptor expression level for both the D1A and D1B receptors. However, this increase is about 3.5-fold steeper (slope factor) for the D1B receptor reflecting a greater agonist-independent activity as compared with the D1A receptor at any receptor expression level. In striking contrast, the relationship between receptor density and the maximal activation obtained through dopamine stimulation of D1A and D1B receptors was inverse. Indeed, maximal activation achieved upon dopamine stimulation was always greater at the D1A receptor. Therefore, activation of D1B receptors leads to a smaller fold stimulation as compared with the agonist activation of D1A receptors. A similar relationship has been shown with the constitutively active mutant β2-AR (7–9).

The observation that the D1B receptor is capable of generating a signal in the absence of agonist occupancy was corroborated further by the following time course data. Overexpression of any G protein-coupled receptor in eukaryotic cells usually leads to an increase in the basal levels of intracellular cAMP in comparison with the mock transfected cells. Such an observation has recently been used for attributing constitutive activity properties to the 5HT2c receptor (28). As shown in Fig. 3, a time course of cAMP accumulation in 293 cells in the absence of agonist reveals that the D1B receptor subtype is able to produce a time-dependent increase in the intracellular cAMP content of 293 cells. Over the same period of time, this accumulation was virtually absent in mock transfected cells and only slightly greater in cells expressing similar levels of human D1A receptors (Fig. 2). This agonist-independent activation of endogenous adenylyl cyclases by the human D1B receptor is consistent with a greater constitutive activity for this receptor subtype. Thus, the data presented in Figs. 1–3 strongly suggest that the D1B receptor has an increased intrinsic ability to interact with G, and to raise the intracellular cAMP level in the absence of agonist when compared with the D1A receptor subtype.

We subsequently investigated the potency of different agonists to stimulate cAMP formation in 293 cells transiently transfected with either the human D1A or human D1B receptor. Fig. 4, upper right panel, shows that dopamine was about 7-fold more potent at the human D1B as compared with the human D1A receptor. Other agonists such as (–)-apomorphine and SKF 38393 were also tested and were approximately 10-
Under the experimental conditions used. Even at a lower expression level (100-400 fmoVmg of membrane protein), it appears that SCH 23390 has increased intrinsic activity at the human D1B receptor compared with the human D1A receptor (Fig. 4, left panels). Similarly, fenoldopam was four times more potent at the human D1B receptor as compared with the human D1A receptor. These results suggest that agonists that stimulate D1B receptors displayed an increased potency for the activation of adenylyl cyclase. Furthermore, all agonists seemed to be full agonists at both receptor subtypes under the experimental conditions used. Even at a lower expression level (100-400 fmoVmg of membrane protein), it appears that SKF 38393 is a full agonist in this cellular system (data not shown). Previous reports have shown that these two agonists can be partial agonists in other cellular systems (29).

The observation of high agonist-independent activity for the D1B receptors prompted us to investigate whether antagonists could regulate the basal activity of cells expressing this D1 receptor subtype. Interestingly, in 293 cells expressing either the D1A or D1B receptors, (-)-butaclamol and flupentixol decreased the basal activities of both receptor to levels similar to those found in mock transfected cells (Fig. 5A). Therefore, in this assay system these two antagonists display negative efficacy (i.e. inverse agonism). This reduction in basal activity appears to be greater in cells expressing the D1B receptor. Surprisingly, the selective D1 antagonist SCH 23390 was a partial agonist at both the human D1A and human D1B receptor, increasing cAMP levels about 2-3-fold above basal levels. Stimulation of adenylyl cyclase was also observed at lower receptor expression (100-400 fmoVmg of membrane protein; data not shown). In this cellular system and under these conditions, it would appear that SCH 23390 has increased intrinsic activity at the human D1B as compared with the D1A receptor (Fig. 5B).
The recent characterization of constitutively active G protein-coupled receptors has led to the elucidation of apparent intrinsic differences in the binding and coupling properties for these two receptors. These observations demonstrate that the naturally occurring D1B receptor subtype displays properties resembling those of constitutively active mutant G protein-coupled receptors (12). These include increased agonist affinity, increased basal activity in the absence of agonist occupancy, increased potency of agonists for stimulating adenyl cyclase, and increased intrinsic activity of partial agonists. Regarding the increased binding affinity for the D1B receptor, (-)-apomorphine and SKF 38393 display small or no differences in comparison with the D1A selective SCH 23390. These different mutants displayed lower affinities at constitutively active mutant G protein-coupled receptors (14). In the work reported here antagonists displayed lower affinities at the D1B than D1A receptor subtype. Although this property of antagonists at constitutively active mutant receptors has been difficult to demonstrate experimentally (7–11), a recent report has shown that β2-AR antagonist ICI 118551 displayed a lower binding affinity and negative efficacy at the constitutively active mutant β2-AR (14).

Interestingly, biochemical and physiological studies of dopaminergic systems have suggested that antagonists such as haloperidol and (+)Ad76 display negative efficacy properties at dopamine D2 and D3 receptor subtypes (32, 33). Furthermore, recent studies have documented antagonist inhibition of the basal signaling activity of the wild type β2-AR, δ-opioid, and 5HT2c receptors (28, 34, 35). This inhibition is achieved by virtue of their negative efficacy activity (30, 31). Taking advantage of such property, Govardhan and Oprian (36) have reported the inactivation of constitutively active mutants of rhodopsin using a series of retinal derivatives designed for potential therapeutic use in the treatment of retinitis pigmentosa. The present results showing that certain receptor subtypes are normally active in the absence of a stimulus and that classical antagonists can reverse this activation may prove to be of physiological and clinical relevance. Antipsychotic drugs used in the management of such diseases as schizophrenia and Tourette syndrome may exert some of their effects by acting via their negative efficacy properties.

In general agreement with properties of constitutively active mutant G protein-coupled receptors, the D1B receptor exhibits an increased potency of agonists for activation of adenyl cyclase. Thus, in addition to the high agonist-independent activity, the increased potency for agonists may have important implications for the biology of the D1B receptor. It is worth noting that further stimulation of D1B receptors can still be observed in the presence of agonists but to a lesser extent than the stimulation obtained through D1A receptors. In contrast, studies done with β2-AR showed that agonist activation of the constitutively active mutant receptor gave a higher maximal stimulation than that obtained through the agonist stimulation of the wild type receptor (9). However, mutagenesis studies performed on the α1B-AR in which Ala360 residue was replaced by all 19 possible amino acid substitutions conferred an increased constitutive activity to all mutant receptors which was accompanied by a wide spectrum of epinephrine-dependent maximal stimulation (8). These different mutants displayed an agonist stimulation that was either decreased, unchanged, or increased in comparison with the wild type. Therefore, it remains unclear if the maximal stimulation obtained through the agonist activation of constitutively active G protein-coupled receptors is related to an increased constitutive activity of the receptor. However, what seems to be consistent with all constitutively active mutant receptors is that the fold increase ("stimulated to basal") is generally lower than for the wild type receptors. As illustrated in Fig. 1A, activation of the human
D1A by 10 μM dopamine results in a 21-fold increase above basal, whereas only a 4-fold increase above basal was observed following a similar dopamine stimulation of the human D1B receptor. At the present time these differences are not easily understood but could potentially result from differential interactions with G proteins. Moreover, it has been shown that in the absence of agonist, constitutively activated mutant α2- and β2-AR were recognized as substrates for the specific β-adrenergic receptor kinase, an interaction that presumably results in a constitutive phosphorylation of these mutant receptors (10, 14, 37). This suggests that constitutively active mutant receptors are partially desensitized even in the absence of agonist occupancy (37). Interestingly, the constitutive desensitization and phosphorylation of the mutant β2-AR could be reversed by the addition of antagonists in the cell culture media (14, 37). These properties of constitutively active receptors may explain the lower fold stimulation by agonists. It will be interesting to examine whether the two D1 receptor subtypes are differentially regulated by second messenger-activated kinases or specific receptor kinases.

Further studies will be needed to delineate the structural determinants involved in the distinct binding and coupling properties of the D1A and D1B receptor subtypes. A body of evidence obtained from mutagenesis studies of the adrenergic receptors have targeted the carboxyl-terminal portion of the third cytoplasmic loop and the cytoplasmic tail as key regions involved in the tight control of the constrained conformation of the receptor in the absence of agonist (7–11). However, it is likely that transmembrane regions of G protein-coupled receptors are also important domains involved in the control of receptor conformation as evidenced by single activating point mutations found in the transmembrane regions of rhodopsin, luteinizing hormone, and melanocyte-stimulating hormone receptors (38–41). Furthermore, a recently uncommon polymorphism of the human β2-AR was identified as a mutation in the fourth transmembrane domain (T164I) which led to a receptor phenotype that displayed converse properties of the constitutively active mutant β2-AR (42). Indeed, this mutant receptor exhibits a decreased affinity for agonists, a decreased potency of epinephrine for stimulation of adenyl cyclase, and a dramatic reduction of the basal activity (42). However, no change in antagonist affinity was observed. Overall, these studies suggest a complex interaction among different domains in the regulation of the active and inactive conformations of receptors.

Recently, several activating mutations have been identified in various G protein-coupled receptors which have been linked to the etiology of numerous conditions (43, 44). For instance, a form of congenital night blindness and retinitis pigmentos is caused by the constitutive activation of rhodopsin by virtue of mutations (G90D, K296E) occurring in its second and seventh transmembrane region, respectively (38–41). Furthermore, a constitutively activating mutation in the sixth transmembrane region of the luteinizing hormone receptor was found to be responsible for the development of male precocious puberty (40). Mutations found in the first intracellular loop and second transmembrane region of the murine melanocyte-stimulating hormone receptor produce a constitutively activated form of the receptor which results in hyperpigmentation of coat color in mice (41). Finally, several somatic mutations have been found in the carboxyl-terminal region of the third cytoplasmic loop and transmembrane regions of the thyroid-stimulating hormone receptor which seem responsible for the development of thyroid adenomas and hyperthyroidism (45, 46). Interestingly, basal levels of cAMP were augmented in cells expressing either luteinizing, melanocyte-stimulating, or thyroid-stimulating hormone-activated mutant receptors when compared with their respective wild type receptors (40, 41, 45, 46).

These different studies have highlighted the pathophysiologic implications of mutations in G protein-coupled receptors. It is still unclear what the physiologic implications are for naturally occurring G protein-coupled receptors with constitutively active phenotypes. However, Lefkowitz et al. (12) have speculated that receptors displaying a greater constitutive activity would have an increased affinity for endogenous agonists associated with a reduced fold stimulation due to the high basal activity. Therefore, these receptors would behave more as on/off switches. In contrast, receptors with a lower constitutive activity (such as the D1A receptor) would be predicted to display a broader spectrum of receptor responses (12). This issue is complicated by the fact that no study has been able to pinpoint the precise localization of the D1B receptor protein, and its quantitation in neurons of the central nervous system as specific tools do not yet exist. Undoubtedly, the tools would help to resolve this issue pertaining the physiological relevance of such properties. However, recent evidence obtained from genetic studies of numerous pathological and physiological conditions have shown that mutations in G protein-coupled receptors with phenotypic 2–4-fold increases in basal activity when compared with their respective wild type receptors appear to be at the basis of these conditions. Likewise, genetically engineered mutations of the α1B-AR which lead to a 2.5-fold increase in the basal activity have been shown to enhance mitogenesis and tumorigenicity in nude mice by acting as protooncogenes (7, 47). Thus, it becomes apparent that “real physiological settings” can sense what might appear to be small differences in the intrinsic coupling properties of G protein-coupled receptors. Therefore, it is tempting to speculate that if these properties are recapitulated in neurons expressing D1B receptors, they may be the basis of an important role in the regulation of some physiological functions of the central nervous system. However, establishment of the real physiological relevance of these observations for cells expressing D1B receptors may have to await the development of subtype selective agonists and antagonists (with negative efficacy properties).

In summary, the present study has delineated differences in the mechanisms underlying the activation of the D1 receptor subtypes. The D1B receptor subtype appears to be the first naturally occurring constitutively active G protein-coupled receptor to be appreciated in vivo. In addition, the different properties for the D1A and D1B receptors may provide an explanation for the existence of multiple dopamine receptor subtypes coupled to the activation of adenyl cyclase.

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