The Journal of Biological Chemistry
Vol. 275, No. 31, Issue of August 4, pp. 23446–23455, 2000
Printed in U.S.A.

Dopamine D5 Receptor Agonist High Affinity and Constitutive Activity Profile Conferred by Carboxyl-terminal Tail Sequence*

Received for publication, January 10, 2000, and in revised form May 8, 2000
Published, JBC Papers in Press, May 11, 2000, DOI 10.1074/jbc.M000157200

Lidia L. Demchyshyn‡§§, Fortunata McConkey‡, and Hyman B. Niznik‡§§***

From the Departments of Psychiatry and Pharmacology and **Institute of Medical Science, University of Toronto, Ontario, Canada M5S 1A8; and the Laboratory of Molecular Neurobiology, Center for Addiction and Mental Health, Toronto, Ontario, Canada M5T 1R8

Dopamine is a neurotransmitter that regulates a variety of physiological functions. Acting through D1- and D2-like receptors, dopamine exerts a major role in regulating neuronal motor control, cognition, event prediction, and emotion and has been implicated in the maintenance and expression of neuropsychiatric disease states such as addiction and schizophrenia (1–5).

In mammals, dopamine D1-like receptors are encoded by two distinct genes, termed D1 (6) and D5 (7), and belong to a superfamily of single polypeptide seven-transmembrane (TM)1 domain receptors that exert their biological effects via intracellular G-protein-coupled signaling cascades. Dopamine D1 and D5 receptors can be distinguished from each other on the basis on a number of inherent attributes (8). These include obvious differences in primary structure, chromosomal localization, and, at an anatomical level, distinct mRNA and cellular and subcellular protein distribution profiles. Despite this, little is known with regard to the molecular determinants that modulate the functional properties of these receptors. This is in large part due to the relatively low expression levels of D5 receptors in neuronal populations and the lack of available pharmacological tools and ligands that can selectively identify this molecule.

When expressed in a variety of mammalian cell lines, a few unique distinguishing pharmacological features between D1 and D5 receptors are evident. The endogenous neurotransmitter dopamine and nonselective dopaminergic agonist 6,7-dihydroxy-2-aminoetetralin (6,7-ADTN) display up to 10-fold higher affinity and efficacy for D5/D1B receptors, whereas nonselective antagonists such as (+)-butaclamol, flupenthixol, and spiperone exhibit much higher affinities for D1/D1A than for D5/D1B receptors. In contrast, the benzazepine class of molecules, in general, does not appear to discriminate between the various D1-like receptor subtypes and may be considered as generic nonselective ligands for all the members of the D1 receptor gene family. These characteristics appear invariant, because cloned vertebrate dopamine D1A and D1B receptors express pharmacological profiles for dopaminergic agonists and antagonists virtually identical to their mammalian counterparts (reviewed in Ref. 9).

* This work was supported in part by grants from National Institute on Drug Abuse, the Medical Research Council of Canada (MT-15581), and the Ontario Mental Health Foundation (to H. B. N.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by an Ontario Mental Health Foundation Fellowship. To whom correspondence should be addressed: Dept. of Discovery Biology and Pharmacology, NPS Allexis Corp., 6850 Goreway Dr., Mississauga, Ontario, Canada L4V 1V7. Tel.: 905-677-0831; Fax: 905-677-9595; E-mail: ldemchyshyn@allelix.com.

1 The abbreviations used are: TM, transmembrane; 6,7-ADTN, (±)-6,7-dihydroxy-2-aminoetetralin; CT, carboxyl-terminal; CY208-243, (±)-4,6,8a,7,8,12β-hexahydro-7-methylindolo[4,3-a]phenanthridine; GPCR, G-protein coupled receptor; NPA, N-propylnorapomorphine; SCH-23390, (R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; SCH-39166, (±)-trans-6,7,7a,9,13β-hexahydro-5-chloro-2-hydroxy-N-methyl-5H-benz[a][2,1]naphthobenzazepine; SKF-38393, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine; SKF-82526, 6-chloro-7,8-dihydroxy-1-(p-hydroxyphenyl)-2,3,4,5-tetrahydro-(1H)-3-benzazepine; wt, wild-type; PCR, polymerase chain reaction.

This paper is available on line at http://www.jbc.org
Although all cloned members of the D1 receptor family stimulate cAMP accumulation, a defining differential characteristic of mammalian D5/D1B receptors is that they exhibit higher levels of constitutive or agonist-independent adenyl cyclase activity, which is inhibited by the inverse agonists (+)-butaclamol and flupenthixol (10, 11). Relative to D1/D1A receptors (12), this characteristic again appears to be inherent to this receptor subtype, because this property is absolutely conserved in all cloned vertebrate members of the D1B receptor subclass (9, 13). To date, unlike that of other naturally occurring constitutively active receptors, which maintain various pathological states (14, 15), the physiological significance of increased basal D5 receptor activity is unknown, but suggests that the D5 receptor may exhibit distinct intrinsic functional correlates relative to D1-like receptors (11).

A number of studies have shown that particular amino acids and regions of both the third intra- and extracellular loop of D1-like receptors play an important role in allowing for the expression of subtype-specific pharmacological and functional attributes of these receptors (reviewed in Ref. 16). In particular, mutation of one conserved amino acid residue (Ile258) in the third cytoplasmic loop of mammalian D1B receptors appears to allow for the expression of agonist-independent receptor activity as well as ligand binding properties (17). In addition, a recent elegant study based on chimeric D1–D5 receptors suggests that regions encoding TM-6/7 and the carboxyl-terminal (CT) tail of D5 and D1 receptors confer the observed unique pharmacological and functional characteristics found in respective cognate wild-type (wt) receptor subtypes (18). Despite these insights, the swapping of relatively large receptor domains obfuscates the contribution of specific regions and amino acid sequence motifs that may confer subtype-specific D1 versus D5 receptor pharmacological and functional profiles.

The carboxyl-terminal region of many G-protein-coupled receptors (GPCRs) has been studied and implicated in regulating many receptor-mediated events, including desensitization, phosphorylation, internalization, and constitutive activity (19–23). Moreover, this region appears to impart functional attributes to a variety of GPCRs by allowing coupling to distinct protein effector systems not classically associated with G-protein activation (24). Structurally, although D1 and D5 display amino acid sequence homology of approximately 80% within transmembrane domains, particular divergence of amino acid sequence identity occurs within the CT tails of these receptors (~30%). Recent work has identified that D5, but not D1, receptor stimulation can regulate ligand-gated channel activity independent of classically defined receptor signaling cascades and this receptor cross-talk is solely dependent upon sequence-specific motifs of the D5-CT tail (25). Although sequences within the CT tail of dopamine D5 receptors allow the functional differentiation of dopamine D5- from D1-like receptors, attempts to define the role of the D5-CT tail in the expression and maintenance of D5 subtype-specific pharmacological and constitutive activity profiles have yet to be made. Here we report, based on a series of D1 and D5-CT tail receptor chimeras, truncation, and deletion mutants, as well as site-directed mutagenesis studies, that the pharmacological and functional signature of the D5/D1B receptor is governed by sequence-specific motifs within the CT tail and that one particular amino acid residue in this region can independently dissociate modifications of D5 receptor constitutive activity from the enhancement of the expressed agonist high affinity pharmacological profile.

**Table 1**

| Receptor/ chimera | Kd (pM) | B_{max} (pmol/mg) |
|-------------------|---------|-----------------|
| D5                | 425 ± 46| 680 ± 106       |
| D1                | 384 ± 32| 1310 ± 185      |
| D5/D1_{CT}        | 540 ± 62| 1125 ± 111      |
| D5/D1D_{CT}       | 348 ± 44| 960 ± 128       |
| D1/D5_{CT}        | 475 ± 53| 840 ± 145       |
| D1/D1D_{CT}       | 391 ± 40| 1095 ± 111      |

**Experimental Procedures**

**Mutant Receptor Construction**

CT-tail Chimeric Receptors—Wild-type human D1 and D5 and vertebrate D1D receptors were used as described previously (6, 7, 26) except that the D1 and D5 receptor constructs were shortened via polymerase chain reaction (PCR; see below) to yield a parental 1.5-kilobase EcoRI/SpeI fragment and subcloned into pCD-ps as described (26). Mutant D1 and D5 chimeric receptors in which the CT tails were swapped were constructed using an overlap PCR extension methodology as described previously (27). Oligonucleotide primers were chosen in the 5′-region upstream to the start site of the parent receptor and the 3′-terminal region downstream of the stop codon of the tail receptor. Primers were also constructed overlapping each other on both DNA strands in a highly conserved region (YAFNADF) downstream of TM7. Approximately 500 ng of cesium chloride-purified DNA containing either human D1, D5, or vertebrate D1D receptors were used as templates, denatured for 5 min at 95 °C, and submitted to 30 cycles of PCR (1 min at 95 °C, 1.5 min at 58 °C, 1 min at 72 °C) with 2.5 units of Vent DNA polymerase (Perkin-Elmer/Cetus) and 500 ng of synthesized specific oligonucleotide primers. The first round of PCR generated two fragments coding the amino-terminal to TM7 of the first receptor and the carboxyl region of the second receptor (D1, D5, or D1D). The amino portion of the D1 receptor was amplified using primers 5′-TCGGAATTC- TCTTTAGGAACTTGGAGGGGT-3′ and 5′-CCGGAAGTCACGATTGAA- GCATA-3′. The amino-terminal of the D5 receptor was generated using specific primers 5′-TGAAGAATTCCTTGAGCGGACCACA-3′ and 5′-CCGGAAGTCAAGATGGGACGCTA-3′. The carboxyl tail of the D1 tail was generated using specific primer 5′-TATGCTTTGATGCT- TGACTTC-3′ and both 5′-TGACTACGTCCTGAGATTGCTGAG- TG-3′ for the D5 receptor, or 5′-GAGAATTTATCTTTTGAGGAATGC- GCAT-3′ for the D1 receptor. Amplified fragments were excised and purified by gel extraction. Aliquots (~500 ng) of the two fragments were combined and subjected to a second round of PCR for 30 cycles as described above, utilizing only the 5′-start site and 3′-stop codon primers, which incorporated EcoRI and SpeI restriction sites, respectively, to facilitate direction subcloning. PCR amplification generated full-length chimeric receptors of ~1.5-kilobase fragments, which were excised, purified, and directionally subcloned into the expression vector pCD-ps.

**D5 Receptor Truncation**—Stepwise truncation mutants of the human D5 receptor CT tail were generated by PCR amplification from the full-length receptor. Primers were constructed in various regions 3′ of the carboxyl tail, and successive shorter fragments were selectively amplified from a full-length D5 receptor template under high-stringency conditions. Eight CT-truncated receptors were constructed using specific oligonucleotide primers 5′-TGAAGAATTCCTTGAGCGGACCACA-3′ and either 5′-CCGGAAGTCAGCATTGAAAGGACTCA-3′ (D5-Phe65), 5′-TGGACTACGTGTTTCGCTTGGCCTACCTC-3′ (D5-Asn388), 5′-TGGACTACGTAAACGGCCTTGGGGACATC-3′ (D5-Val145), 5′-TGGGA- CTAGTCTGTGGTGGTGCACCTC-3′ (D5-Glu429), 5′-TGGGAATTTACGTCATTGGGACGACATGGATGAA-3′ (D5-Glu439), 5′-TGGGAATTTACGTCATTGGGACGACATGGATGAA-3′ (D5-Ile446), or 5′-TGGGAATTTACGTCATTGGGACGACATGGATGAA-3′ (D5-Asp454). Approximately 500 ng of DNA plasmid containing the...
Inhibitory constants ($K_i$) of various dopaminergic agonists and antagonists for $[^{3}H]SCH$-23390 binding to membranes prepared from COS-7 cells transfected with the human D1 and D5 receptor genes and D1/D1CT, D5/D1CT, D5/D1DCT, and D5/DIDCT chimeras are listed in order of potency for the D5 receptor. Values represent the means of three to six independent experiments, each conducted in duplicate with estimated $K_i$ values varying less than 15%. $K_i$ values for discriminating D1 and D5 receptor agonists and antagonists are shown in boldface.

| Table II Dissociation constants ($K_i$) for the binding of dopaminergic compounds to wild-type and chimeric D1 and D5 receptors |
|-----------------|------------------|-----------------|-----------------|
| **Agnostins**   | **D5** | **D5/D1CT** | **D5/DIDCT** | **D1** | **D1/DCT** | **D1/DIDCT** |
| SKF-82526       | 5     | 15            | 10             | 27     | 17           | 28           |
| SKF-38395       | 34    | 126           | 211            | 196    | 140          | 319          |
| Liarudine       | 96    | 572           | 452            | 60     | 49           | 60           |
| CY208–243       | 40    | 596           | 574            | 417    | 280          | 366          |
| Apomorphine     | 125   | 661           | 908            | 827    | 386          | 714          |
| Dopamine        | 189   | 3305          | 3520           | 3340   | 1320         | 3590         |
| NPA             | 369   | 4610          | 4690           | 1630   | 876          | 2570         |
| 6,7-ADTN        | 337   | 6800          | 6755           | 4540   | 2955         | 3730         |
| **Antagonists** |        |                |                |        |              |              |
| SCH-23930       | 0.452 | 0.375         | 0.358          | 0.368  | 0.366        | 0.515        |
| SCH-39166       | 0.392 | 0.755         | 0.320          | 0.181  | 0.727        | 0.276        |
| Fluoxetine      | 0.213 | 11            | 8              | 3      | 5            | 6            |
| Butaclamol      | 14    | 15            | 13             | 2      | 3            | 2            |
| Chlorpromazine  | 75    | 109           | 77             | 27     | 37           | 40           |
| Haloperidol     | 95    | 155           | 41             | 76     |              |              |
| Spiperone       | 3500  | 7000          | 344            | 215    |              |              |

**Compound**

| SCH-23390 | 0.452 | 0.375 | 0.358 | 0.368 | 0.366 | 0.515 |
| SCH-39166 | 0.392 | 0.755 | 0.320 | 0.181 | 0.727 | 0.276 |
| Fluoxetine | 0.213 | 11    | 8     | 3     | 5     | 6     |
| Butaclamol | 14    | 15    | 13    | 2     | 3     | 2     |
| Chlorpromazine | 75    | 109   | 77    | 27    | 37    | 40    |
| Haloperidol | 95    | 155   | 41    | 76    |       |       |
| Spiperone   | 3500  | 7000  | 344   | 215   |       |       |

**Dissociation constants ($K_i$) for the binding of dopaminergic compounds to wild-type and chimeric D1 and D5 receptors**

**Cyclic AMP Accumulation Assay**

COS-7 cells were transiently transfected as described above, placed in 24-well plates, and grown for 72 h. Cells were washed with 0.5 ml of prewarmed Dulbecco’s a minimal essential media containing 1-methyl-3-isobutylxanthine and 1 μM propranolol, then incubated in the above media in the presence or absence of agonist or inverse agonist for 15 min at 37°C and 5% CO₂. Increasing concentrations of dopamine or agonists were added, and the cells were incubated for an additional 15 min at 37°C and 5% CO₂. The reaction was terminated by the addition of 0.5 ml of 0.2 M HCl and incubation for 20 min at 4°C. Cellular debris was pelleted, and aliquots of the supernatant were used to determine the cAMP content via immunodetection (Amersham Pharmacia Biotech) as described previously (26). Estimated EC₅₀ and IC₅₀ values were obtained as described previously. To ensure equivalence of whole cell CAMP assay comparisons, receptor densities were monitored for $[^{3}H]SCH$-23390 binding (3.0 nM final concentration) and $[^{3}H]mutant$ or $[^{3}H]chimeric$ receptor preparations. Data were analyzed using a two-tailed t test set at 0.1 level of significance.

**RESULTS AND DISCUSSION**

To determine what potential role sequences within the highly sequence divergent carboxyl tails may play in the pharmacological and functional differentiation of the human D1 and D5 receptors, D1/D5 receptor chimeras were generated in which the CT tail of the parent receptor was removed and substituted by complementary CT-tail receptor sequences encoded by other members of the mammalian/vertebrate D1 receptor gene family. Dideoxynucleotide sequence analysis confirmed the absence of any spurious PCR-generated sequence mutations either within the CT-tail splice site or throughout the entire full-length mutant receptor sequence.

We first assessed the effects of swapping the CT tails of...
concentration) binding was 0.55, 0.78, and 0.69 pmol/mg of protein for D5, D1, and D5/D1 CT receptors, respectively. Estimated inhibitory constants (Ki) for these compounds are listed in Table I. Data are representative of at least three independent experiments, each conducted in duplicate. C and D, correlation plots estimating Ki values of various dopaminergic (C) agonists and (D) antagonists to inhibit [3H]SCH-23390 binding to the human wt (C) D1 versus D1/D5 CT and (D) D5 versus D5/D1 CT chimeric receptors. Ki values were taken from Table II. The line of identity or equimolarity is also drawn. Substitution of the D5-CT tail significantly reduced agonist, but not antagonist Ki values, respectively (p < 0.05).

As summarized in Table II, wt D5 binds the endogenous neurotransmitter dopamine and some other dopamine agonists such as 6,7-ADTN with 10-fold higher affinity than D1 while displaying a 2- to 7-fold preference for dopaminergic agonists such as apomorphine, N-propynorapomorphine (NPA)-, and benzazepine-like compounds, respectively. With regard to antagonists, D1 receptors in contrast display somewhat higher affinity for non-benzazepine antagonist compounds, particularly butaclamol and flupenthixol. To assess the potential contribution of sequence motifs within the CT tail to the overall pharmacological differentiation of human D5 versus D1 receptors, we analyzed the ability of numerous dopaminergic agonists and antagonists to inhibit the binding of [3H]SCH-23390 to generated receptor CT tail substitution mutants relative to parental wt D1 and D5. Replacement of human D5-CT sequence with amino acids encoded by the D1 receptor CT tail resulted in a mutant receptor in which [3H]SCH-23390 binding was inhibited in a concentration-dependent and uniphasic manner by a variety of dopaminergic agonists with a rank order of potency and pharmacological profile comparable to that exhibited by the human D1 receptor. Thus, as illustrated in Fig. 1A, D5/D1 CT receptor mutants exhibited estimated Ki values for discriminating agonist dopamine 10-fold lower than wt D5 receptors and virtually identical to Ki values displayed for these compounds by the human D1 receptor. Similar reductions in affinity for other discriminating dopaminergic agonists, such as 6,7-ADTN and apomorphine, were noted with estimated Ki values in Table II. The effects of CT-tail substitution are specific for D1-like agonist compounds, because the pharmacological profile and estimated affinities for numerous antagonist ligands, including D1/D5 discriminating agents such as butaclamol and flupenthixol, were retained by mutant receptors relative to wt receptors (see Fig. 1B) consistent with estimated Ki values for [3H]SCH-23390 binding to these mutants (Table I). Estimated Ki values for a number of antagonists for this particular chimera are listed in Table II. To determine if the selective loss of agonist high affinity by the D5/D1 CT mutant receptor was due to the removal of sequence motifs encoded by the D5 receptor tail or a result of the addition of specific D1-CT sequence domains that may confer inherent D1 agonist low affinity characteristics, we analyzed the pharmacological profiles of a D5 receptor mutant in which the CT tail was substituted with highly divergent but corresponding sequences of the vertebrate D1D receptor (26). The dopamine D1D receptor displays an overall pharmacological
profile and affinity for dopaminergic agonists similar to that of the human D5 receptor. Despite differences in overall length and net charge, the generated D5/D1D_CT chimera displayed agonist affinity profiles identical to that exhibited by either the wt human D1 receptor or D5/D1_CT mutant with a loss of affinities for the agonists dopamine, 6,7-ADTN, NPA, and apomorphine greater than 10-fold, whereas SKF-38393 and SKF-82526 only displayed a 2-fold loss in affinity. As with the human D5/D1_CT mutant described above, the D5/D1_CT receptor exhibited antagonist binding profiles identical with that observed for wt D5 receptors with estimated \( K_{i} \) values listed in Table II. These data suggest that it is the removal of CT sequence motifs of the D5 receptor, rather than the addition of D1- or D1D-CT-tail sequence domains, that allow for the expression of agonist D1 receptor characteristics and is consistent with the notion that the pharmacological profile of the D1/D1A receptor is an inherent default characteristic of members of the D1 receptor family (9, 13, 27).

The contention is further supported by the fact that sequence motifs encoded within the CT tail of the D5 receptor cannot fully confer D5 receptor pharmacological characteristics to other members of the D1 receptor family. As outlined in Table II, the D1/D5_CT chimera retained a pharmacological profile consistent with that observed for the wt D1 receptor for both agonists and antagonists, although agonist potencies were observed to slightly, albeit nonsignificantly, shift to the left. Similar results were obtained with the D1/D1D_CT receptor mutant in which the substitution of D1-CT tail with that encoded by the D1D receptor did not affect either agonist or antagonist potencies relative to wt D1 or mutant D1/D5_CT receptor (Table II). The selective modification of D5 agonist high affinity binding interactions by swapping the CT-tail sequence but not that of antagonists or both agonists and antagonists at D1 receptors is depicted graphically in Fig. 1, C and D. Correlation plots of estimated \( K_{i} \) values for agonists obtained at wt D5 versus D5/D1_CT chimeric receptors significantly deviate from unity and are distinct from that observed for D1 versus D1/D5_CT tail mutants, which exhibit a virtual one-to-one correspondence in both estimated agonist and antagonist affinity values (Fig. 1C). Thus, estimated \( K_{i} \) values for agonists but not antagonists at D5 versus D5/D1_CT chimeric receptors (Fig. 1D) significantly diverge from this one-to-one correspondence in affinity values (Z value of 2.9, \( p < 0.05 \)). The inability to reciprocally confer D5 receptor characteristics to D1 receptors by D5-CT sequences suggests that additional D5 receptor domains must be present for the full expression of its pharmacological signature to occur, a finding similar to that previously reported for D1/D5 receptor substitution mutants within the third cytoplasmic loop (17). These may include domains encoded by the last two transmembrane domains and third extracellular loop (18). In any event, the data suggest that CT-tail substitution alone has little effect in altering D1 receptor pharmacological profiles and agonist affinity characteristics unlike that observed for the human D5 or the vertebrate D1B receptors (27).

To assess whether the observed selective reductions in D5 agonist ligand binding affinity by CT-tail substitutions were functionally relevant, we examined the ability of D5/D1_CT mutant receptors to stimulate cAMP accumulation constitutively and in response to agonist stimulation. Transfection protocols were optimized to maintain comparably similar levels of expression between wt and chimeric receptors. Although previous studies have demonstrated the importance of amino acids in the third cytoplasmic loop and regions encoding TM6 to the receptor carboxyl terminus for the maintenance and expression of constitutive nature of the D5 receptor (17, 18, 28), we show here that the replacement of the D5 receptor CT tail alone converts D5 receptor agonist-independent stimulation of cAMP accumulation and responsivity to the inverse agonists butaclamol and flupenthixol to one displayed by wt D1 receptors. As illustrated in Fig. 2A, constitutive activity generated by the wt D5 receptor was approximately 2- to 3-fold higher relative to basal cAMP levels generated by wt D1 receptors under similar levels of expression and consistent with previous observations (10, 17, 18). Basal cAMP levels generated by either D1 or D5 receptors were blocked by the inverse agonist butaclamol (10 \( \mu M \)) and flupenthixol (not shown) and not by SCH-23390 (10 \( \mu M \)). D5/D1_CT receptor mutants resulted in the significant reduction of constitutive basal cAMP activity and responsivity to inverse agonists resembling levels and activity inherent of wt D1 receptors. Similar results were obtained with D5/D1D_CT receptor mutants (data not shown). In contrast, and consistent with ligand binding data described above, replacement of the D1-CT tail with that of the D5-CT tail did not significantly effect mutant receptor constitutive basal cAMP accumulation or responsiveness to inverse agonists and displayed characteristics virtually identical to wt D1 receptors. These data again suggest that dopamine D5-CT sequence motifs alone cannot confer functional D5 receptor characteristics to D1-like receptors.

We next assessed the ability of dopamine to maximally stimulate cAMP in both wt and mutant chimeric receptors, because previous work has indicated differences in the potency of agonist-mediated coupling profiles of D1 and D5 receptors (10, 17, 18). Under the present experimental conditions, dopamine stimulated maximal whole cell cAMP accumulation by both wt D1, wt D5, and CT-tail chimeric receptors to virtually identical basal levels the effects of which were blocked by the D1/D5 receptor antagonist SCH-23390 (Fig. 2B). These data suggest that sequences encoded by the CT tails of D5 or D1 receptors do not influence the intrinsic activity of dopamine at these receptors similar to that seen with vertebrate D1A/D1B_CT-tail substitution mutants (27) but distinct from that observed with D1 and D5 receptors in which other sequence domains have been swapped or replaced (17, 18, 28, 29). We next assessed dose-response curves for dopamine-stimulated whole cell cAMP accumulation by both wt D1, wt D5 and CT-tail chimeric receptors. These are illustrated in Fig. 2C, and as previously reported, the potency of dopamine is ~3-fold higher for dopamine D5-mediated cAMP accumulation than for that with D1 with estimated \( EC_{50} \) values as listed in Table III. The selective reduction of D5 receptor constitutive activity by replacement of its CT tail by D1-CT sequence was accompanied by a concomitant but small rightward shift (~2-fold) in the potency for dopamine-stimulated cAMP accumulation with an estimated \( EC_{50} \) value somewhat intermediate to that observed for wt D1 and D5 receptors and without any change in the maximal value of cAMP production. These data suggest that, in addition to the

### Table III

| Compound | D5 | D5/D1_CT | D1 | D1/D5_CT |
|----------|----|----------|----|----------|
| **EC\(_{50}\)** (nM) | | | | |
| Dopamine | 364 ± 9 | 700 ± 141\(^a\) | 1230 ± 202 | 655 ± 180\(^a\) |
| 6,7-ADTN | 225 ± 68 | 278 ± 70 | 479 ± 81 | 344 ± 49 |
| SKF-82526 | 108 ± 43 | 242 ± 67 | 244 ± 50 | 144 ± 43 |
| SKF-38393 | 329 ± 53 | 392 ± 72 | 531 ± 79 | 372 ± 67 |

\(^a\) Estimated \( p < 0.05 \) when compared to wt controls.
observed loss of agonist high affinity binding characteristics and constitutive activity profiles, D5 receptor mutants display functional properties more reminiscent of \textit{wt} D1 receptors following substitution of CT-tail sequences with corresponding sequences of either D1 or D1D receptors. Similarly, as listed in Table III, D1/D5\textsubscript{CT} receptor chimeras displayed an apparent ~2-fold enhancement in the potency of dopamine to stimulate cAMP accumulation relative to \textit{wt} D1 receptors, albeit, with estimated EC\textsubscript{50} values intermediate to that exhibited by D1 and D5 receptors and not significantly distinct from D5/D1\textsubscript{CT} receptor mutants. The fact that sequence motifs on either D1- or D5-CT tails do not fully confer or reconstitute dopamine agonist-promoted coupling of D1/D5\textsubscript{CT} or D5/D1\textsubscript{CT} receptor mutants to mimic \textit{wt} D5 or D1 receptors, respectively, would again suggest that additional molecular determinants are necessary for the full expression of both D1 and D5 dopamine-mediated receptor coupling (18) but not for exhibited D5 ligand binding and constitutive activity profiles. This notion is further attested to by the observation that the observed shifts in the potency for dopamine-stimulated cAMP accumulation with CT-tail chimeric receptors appear specific to the endogenous neurotransmitter dopamine itself, because results obtained for the discriminating agonist 6,7-ADTN and both SKF-82526 and partial agonist SKF-38393 revealed no consistent modification in the estimated EC\textsubscript{50} values of these compounds at \textit{wt} versus chimeric receptor (Table III). Thus we conclude that despite the trend for D5/D1\textsubscript{CT} and D1/D5\textsubscript{CT} chimeric receptors to display EC\textsubscript{50} values for dopamine-stimulated cAMP accumulation (Table III), more reminiscent of those exhibited by \textit{wt} D1 and D5 receptors, respectively, these are particular for the endogenous neurotransmitter and that CT-tail sequences cannot alone impart dopamine-mediated receptor coupling characteristics significantly different from their cognate receptors. Moreover, these data suggest that the reported hallmarks of constitutively active mutant or native receptor agonist-independent activity, increased binding affinity and functional potency for agonists as well as lower affinity for antagonists/inverse agonists (14, 15), all characteristics of the naturally occurring dopamine D5 receptor, can be dissociated from each other by sequences encoded within the D5-CT tail. As such, it appears that only constitutive activity and agonist but not antagonist ligand binding profiles are maintained by sequences within the D5-CT tail. Full expression of dopamine D5 functional agonist potencies and modification of antagonist/inverse agonist binding affinities, and possibly potencies, would require the participation of additional molecular determinants and amino acid domains as attested to in the literature for other GPCRs (19).

To identify particular amino acid domains within the CT tail of the D5 carboxyl tail that are critical to both its expressed pharmacological profile and agonist-independent functional signature, a series of CT-truncated D5 receptor mutants was constructed. As schematically illustrated and summarized in Fig. 3A, the shortest construct was terminated at amino acid 365 (D5-Phe\textsubscript{365}) following the conserved region distal to TM7. Each subsequent stepwise mutant (D5-Asn\textsubscript{388}, D5-Val\textsubscript{418}, D5-Asp\textsubscript{448}) was constructed to contain an additional 30 amino acids from the previous truncation. When transiently expressed in COS-7 cells, however, these truncated D5-Phe\textsubscript{365}, D5-Asn\textsubscript{388}, and D5-Val\textsubscript{418} receptors displayed very poor or a total lack of ability to bind \textsuperscript{3}H\textsubscript{H}SCH-23390 (Fig. 3; A and B) consistent with previous results on the effects of CT substitution/deletion mutants of the D1 receptor (see Refs. 16 and 29) and possibly as a result of poor receptor processing or traffic-

\[ 0.902 \pm 0.177; \text{ and D1, } 1.0 \pm 0.150 \text{ in pmol/mg of protein as indexed by } \text{[H]} \text{SCH-23390 binding (3.5 nm, final concentration).} \]
Dopamine D5 receptor pharmacological and functional signature of D5 receptors (D5-Phe365, D5-Asn388, D5-Val418, D5-Glu429, D5-Phe438, D5-Asp448) and a synopsis of the data obtained with these mutant receptors relative to wt receptors and a synopsis of the data obtained with these mutant receptors relative to wt receptors.

Results and Discussion.

The pharmacological and functional characteristics of D5 receptors (D5-Phe365, D5-Asn388, D5-Val418, D5-Glu429, D5-Phe438, D5-Asp448) and a synopsis of the data obtained with these mutant receptors relative to wt receptors is illustrated in Fig. 3. The D5 truncation mutant D5-Asp448 displayed basal cAMP levels, responsiveness to the inverse agonist butaclamol, and maximal dopamine-stimulated cAMP accumulation identical to wt D5 receptors (Fig. 3C). It is of interest to note, however, that the D5-Asp448 receptor mutant displayed some inverse agonist activity to SCH-23390 unlike wt D5 receptors and suggests that the CT tail and surrounding regions of D1 and D5 receptors, under certain conditions, influence the expression of inverse maximal dopamine (10 \( \mu M \))-stimulated cAMP (+ indicates at least 70% of control wt values calculated following subtraction of basal activity); and agonist-independent cAMP accumulation (basal) and that inhibited by butaclamol (also see C below). B, restoration of D5 receptor ligand binding characteristics by the D5 truncation mutant D5-Asp448. COS-7 cells expressing wt D5 and various CT tail truncation mutants were incubated with the indicated concentrations of dopamine or butaclamol and assayed for \( [3H] \)SCH-23390 (~350 pm) binding activity. Estimated \( K_i \) values for these and other compounds at the D5-Asp448 receptor are listed in Table IV. All other CT truncation mutants displayed extremely low levels of specific \( [3H] \)SCH-23390 binding. C, maximal dopamine-stimulated and constitutive whole cell cAMP production by various D5-CT truncation mutants. Basal and values for maximal cAMP accumulation with 10 \( \mu M \) dopamine are given as well as whole cell basal cAMP accumulation in the presence of 10 \( \mu M \) SCH-23390 or butaclamol. D5 receptor agonist-independent activity and response to inverse agonists is lost with the truncation of the D5 carboxyl tail (\( p < 0.01 \)) while still responsive to maximal stimulation by dopamine to 77% of control (basal subtracted) with estimated EC\(_{50}\) values for dopamine listed in the text. Only the D5-Asp448 truncation mutant displays functional characteristics virtually identical to wt D5 receptors. Results shown are means ± S.E. of four to six independent experiments, each conducted in duplicate. D, dose-dependent inhibition of dopamine-stimulated whole cell cAMP accumulation by SCH-23390 in wt and D5-CT-truncated mutants, D5-Asn388 and D5-Val418. Maximal whole cell cAMP production was measured by pretreatment of cells expressing D5 and D5-CT mutants with increasing concentrations of SCH-23390 followed by the addition of 10 \( \mu M \) dopamine. Values are expressed as percentage of maximal response obtained by either wt or truncated mutants after subtracting basal activity as described above. Data shown are representative of three independent experiments, each conducted in duplicate with estimated IC\(_{50}\) values for SCH-23390 described under "Results and Discussion."
Pharmacological and Functional Signature of D5 Receptors

Inhibitory constants ($K_i$) of various dopaminergic agonists and antagonists for $[^3H]$SCH-23390 binding to wild-type D1 and D5 receptors and various mutant receptors expressed in COS-7 cells

| Compound | D5 | D5-trunc\(^a\) Gln\(^{439}\) D5 Gln\(^{439}\) del | D5 Gln-Ala\(^{439}\) | D5 Gln-Ile\(^{440}\) | D1 | D1/D5 Gln\(^{439}\) del | D1/D5 Gln-Ala\(^{439}\) |
|----------|----|-------------------|------------------|------------------|----|-----------------|-----------------|
| Agonists |    |                   |                  |                  |    |                 |                 |
| SKF-82526 | 8  | 4                | 5                | 4                | 6  | 29              | 26              |
| SKF-38393 | 62 | 39               | 35               | 51               | 55 | 110             | 265             |
| Apomorphine | 125 | 81              | 46               | 56               | 79 | 763             | 386             |
| Dopamine  | 220 | 318             | 50               | 62               | 2770| 3400           | 2100            |
| NFA       | 429 | 373             | 165              | 144              | 138 | 1510           | 1800            |
| 6,7-ADTN  | 568 | 390             | 96               | 91               | 88  | 4400           | 3200            |
| Antagonists |    |                   |                  |                  |    |                 |                 |
| SCH-23390 | 0.401 | 0.272          | 0.319            | 0.354            | 0.654 | 0.351          | 0.555           |
| Flupenthixol | 9   | 11              | 6                | 6                | 3   | 1              | 3               |
| Butaclamol | 18  | 21              | 17               | 15               | 17  | 2              | 1               |
| Chlorpromazine | 75 | 64              | 38               | 28               | 45  | 37             | 18              |
| Haloperidol | 81  | 45              | 44               | 47               | 51  | 40             | 35              |

\(^a\) trunc, truncated.  \(^b\) del, deleted.

Table IV

$K_i$ values for dopaminergic agonists and antagonists for $[^3H]$SCH-23390 binding to wild-type D1 and D5 receptors and various mutant receptors expressed in COS-7 cells

Agonism (also see Refs. 10, 18, 27, 30). Moreover, the D5-Asp\(^{448}\) truncation mutant expressed an estimated EC$_{50}$ for dopamine-stimulated cAMP accumulation identical to that of wt D5 receptor (390 ± 87 versus 440 ± 98 nM) and distinct from that of wt D1 receptor and D5-Phe\(^{365}\) truncation mutants (1890 ± 512 versus 2100 ± 499 nM).

A further series of CT-tail truncation mutants of 10 amino acids each was generated to pinpoint those amino acid motifs within positions 418 and 448 that reconstituted D5 receptor characteristics. These truncated mutants, termed D5-Glu\(^{429}\) and D5-Phe\(^{438}\), similar to D5-Phe\(^{365}\), D5-Asn\(^{468}\), and D5-Val\(^{418}\) did not display $[^3H]$SCH-23390 ligand binding characteristics or constitutive activity but did exhibit dopamine-stimulated cAMP accumulation (data not shown). These data suggest that amino acids 438–448 are absolutely required to allow for the pharmacological and functional attributes that distinguish it from wt members of the D1 receptor family, and that the last 30 amino acids of the D5 receptor do not partake in these events. These amino acids are thought to confer additional functional attributes to the D5 receptor, namely, the modulation of $\gamma$-aminobutyric acid (type A) receptor-mediated ligand-gated channel activity (25).

Alignment of the amino acid sequence of all available cloned and pharmacologically characterized members of the vertebrate D5/D1B receptor between D5-Phe\(^{365}\) and D5-Asp\(^{448}\) reveals significant sequence variability in this area. As depicted in Fig. 4A, only one amino acid (Gln) is absolutely conserved between all cloned members of this receptor subclass. To assess whether Gln\(^{439}\) is involved in either the expression and/or maintenance of inherent D5 receptor pharmacological and functional characteristics, we first constructed D5-CT-truncation tail mutants terminating at Gln\(^{439}\) or the following amino acid D5-CT-tail truncation mutants terminating at Gln\(^{439}\) or I\(^{440}\) bound $[^3H]$SCH-23390 with high affinity (500 ps, see Table V) and displayed pharmacological profiles and estimated $K_i$ values for dopamine and butaclamol (Fig. 4, B and C) as well as other dopaminergic agonists and antagonists (listed in Table IV) indistinguishable from that observed for wt D5 receptors. Similarly, these truncation mutants increased cAMP production following stimulation with agonists such as dopamine to levels equivalent to wt D5 receptors. As depicted in Fig. 4D, enhanced constitutive basal cAMP accumulation rates and sensitivity to the inverse agonist butaclamol were observed, similar to that of wt D5 receptors despite the fact that Gln\(^{439}\)/Ile\(^{440}\) truncation mutants displayed relative poorly expression rates or $B_{max}$ values (Table V). These data suggest that the addition of just one amino acid to truncated D5-Phe\(^{365}\) receptor mutants (which display functional characteristics of D1-like receptors and no $[^3H]$SCH-23390 ligand binding activity; see above) fully restores all the pharmacological and functional characteristics inherent in wt D5 receptors.

To assess the relative contribution of Gln\(^{439}\) to the expressed pharmacological and functional profile of human D5 receptors, we constructed full-length D5 receptor mutants in which Gln\(^{439}\) was deleted or substituted with alanine or isoleucine. D5 receptor site-directed mutants in which either Gln\(^{439}\) was deleted or substituted by Ala or Ile bound $[^3H]$SCH-23390 with high affinity and estimated receptor densities not significantly different from wt D5 receptors (Table V). In contrast to expectation, these receptor mutants displayed agonist affinity values for discriminating agonists from 3- to 6-fold higher than that exhibited by wt receptors. Thus, as depicted in Fig. 4B, dopamine inhibited the binding of $[^3H]$SCH-23390 to these mutants in a concentration-dependent and uniphasic manner with estimated $K_i$ values of ~60 nM. Similar results were obtained for other D1 versus D5 discriminating agonists, including 6,7-ADTN and NPA (~3 fold) but not for benzazepine-like agonist compounds (see Table IV). Antagonist affinities, including that of butaclamol, remained unchanged (Fig. 4C). All estimated $K_i$ values for these and other dopaminergic compounds at various single amino acid D5 receptor deletion/substitution mutants are listed in Table IV. These data suggest that the highly conserved D5/D1B receptor residue Gln\(^{439}\) exerts a tonic negative modulatory influence on the pharmacological signature of native D5 receptors, which without this braking influence can display "super" D5 characteristics with expressed affinities for discriminating agonists ~5 fold higher than wt D5 receptors. Again, as with the D1/D5 CT mutants described above, D1/D5 CT chimeras expressing the mutant Gln\(^{439}\) deletion or substitution sequence cannot confer super D5 receptor pharmacological characteristics to members of the D1 receptor family. As outlined in Table IV, the D1/D5 Gln\(^{439}\) deleted or D1/D5 Gln-Ala\(^{439}\) full-length mutant chimera retained a pharmacological profile consistent with
Estimated maximal receptor density ($B_{\text{max}}$) and dissociation constants ($K_d$) of $[^3H]$SCH-23390 binding to wild-type and various mutant receptors

| Receptor/mutant | $K_d$ ($\mu M$) | $B_{\text{max}}$ (pmol/mg) |
|----------------|-----------------|-----------------------------|
| D5 wt          | 415 ± 68        | 739 ± 141                   |
| D1            | 384 ± 54        | 1316 ± 237                 |
| D5-Gln$^{439}$ | 540 ± 81        | 180 ± 37                   |
| D5-Ile$^{440}$ | 345 ± 55        | 156 ± 30                   |
| D5-Ile$^{440}$del | 475 ± 73    | 1080 ± 173                 |
| D5-Gln-Ala$^{439}$ | 487 ± 97     | 1020 ± 185                 |
| D5-Gln-Ile$^{439}$ | 654 ± 104    | 990 ± 138                   |
| D1/D5-Gln$^{439}$ del | 724 ± 130   | 302 ± 58                   |
| D1/D5-Gln-Ile$^{439}$ | 596 ± 89    | 270 ± 54                   |

Pharmacological and Functional Signature of D5 Receptors

Estimated maximal receptor density ($B_{\text{max}}$) and dissociation constants ($K_d$) of $[^3H]$SCH-23390 binding to membranes prepared from COS-7 cells transfected with the human D1 and D5 receptor genes or CT-truncated, -deleted, and -substituted mutants. Values represent the means of three independent experiments each conducted in duplicate.

that observed for the wt D1 receptor for both agonists and antagonists. The inability to reciprocally confer D5 or super D5 receptor characteristics to D1 receptors by D5-CT sequences suggests, as above, that additional D5 receptor domains indeed partake in the full expression of its pharmacological signature. The full complement of these expressions has yet to be elucidated.

In any event, to assess whether enhanced agonist affinity values for these mutant receptors are associated with corresponding augmentation of functional receptor characteristics, we analyzed the basal and dopamine-stimulated cAMP accumulation as well as the expression of inverse agonist activity exhibited by these receptors. As depicted in Fig. 4D, relative to wt D5 receptors (see Figs. 2A and 3C), there is no concomitant increase in the intrinsic activity of agonist-independent basal cAMP accumulation or responsivity to inverse agonists. Likewise, D1/D5-CT-Gln$^{439}$-deleted or D1/D5-CT-Gln-Ala$^{439}$ full-length mutant chimeric receptors displayed functional characteristics not significantly different from that of wt D1 receptors (data not shown). These data clearly suggest that, in addition to well characterized receptor and G-protein binding domains, the pharmacological and functional signature of the D5/D1B receptor is modulated by sequence-specific motifs within the CT tail and that single amino acid residues within this region can independently modulate D5 receptor constitutive activity from its expressed agonist high affinity pharmacological profile.

To date, although many studies have addressed the roles played by various amino acids and sequence-specific domains within the D1 receptor that relate to both its expressed pharmacology and overall function (16), few have examined the issue of which molecular determinants govern and allow for the discrimination of human D5 from D1 receptors. In summary, we conclude: 1) that a region exists within the CT tail of the dopamine D5 receptor that allows for the full expression of two of the major distinguishing pharmacological and functional features inherent of this subclass, namely, higher agonist affinities and constitutive activity relative to the D1 receptor family; 2) that the last 40 amino acids of the D5 receptor are not necessary for its observed pharmacological and functional signature or expression; 3) a highly conserved amino acid within the D5/D1B receptor subclass can further enhance and modulate agonist ligand binding affinities but independently main-
tain normative constitutive activity profiles; 4) that sequences within the CT tail confer the receptors ability to bind $[^3]$H]SCH-23390 with high affinity, yet conserve its ancient functional status as a dopamine-stimulating adenylyl cyclase receptor (31); and 5) that these sequence motifs alone cannot fully impart to other members of the D1 receptor subfamily characteristics attributable to the D1B/D5 receptor.

Clues to the possible functional differentiation of D1- and D5-like receptors have only recently begun to be unraveled. These have been based primarily on data obtained from targeted gene mutations of D1 or D5 receptors (see Refs. 32–34), the evolutionary history of these genes (31), and the observed acquisition of neural expression territories and cellular and subcellular distribution profiles (35–37) of these receptors in specific neural populations. The data presented here suggest that, despite the observed functional redundancy of multiple members of the D1-like receptor gene family, which include also the D1C and D1D receptor family in vertebrates, the highly variable amino acid sequence motifs encoded within the CT tails of these receptor subtypes can impart highly selective and exquisite subtype-specific receptor functions and suggest that members of the D1-like gene family are not fully capable of reconstituting each other's function. Given the existence of D5 receptor point mutations (38), some of which may influence agonist high affinity binding reactions (39) and be associated with specific behavioral manifestations of neuropsychiatric disease states (40), further work on the role these CT sequences may play in the expression of subtype-specific dopamine D1 versus D5 receptor-mediated events in both health and disease appears warranted.

REFERENCES

1. Beringer, R. J., and Miller, R. (1997) *Neurosci. Biobehav. Rev.* 21, 335–345
2. Schultz, W., Daylan, P., and Montague, R. R. (1997) *Science* 275, 1593–1599
3. Baldessarini, R. J. (1997) in *The Dopamine Receptors* (Neve, K. A., and Neve, R. L., eds) pp. 457–498, Humana Press, Totowa, NJ
4. Goldman-Rakic, P. S., and Selemon, L. D. (1997) *J. Neurosci.* 17, 1593–1599
5. Niznik, H. B., Liu, F., Sugamori, K. S., Cardinaud, B., and Vernier, P. (1998) *J. Biol. Chem.* 273, 425–432
6. Demchyshyn, L. L., Sugamori, K. S., Lee, F. J. S., Hamadanizadeh, S. A., and Niznik, H. B. (1995) *J. Biol. Chem.* 270, 4005–4012
7. Cardinaud, B., McGill, J. M., Guibert, B., Sugamori, K. S., Liu, F., Niznik, H. B., and Vernier, P. (1999) *J. Biol. Chem.* 274, 10999–11006
8. Abadji, V., Lucas-Lenard, J. M., Chin, C., and Kendall, D. A. (1999) *J. Neurochem.* 72, 2032–2038
9. Mary, S., Gomeza, J., Preziale, L., Bockhart, J., and Pin, J.-P. (1998) *J. Biol. Chem.* 273, 425–432
10. Namba, T., Sugimoto, Y., Negishi, M., Irie, A., Ushikubi, F., Kakizuka, A., He, S., Ichikawa, A., and Narumiya, A. (1993) *Nature* 365, 166–170
11. Hall, R. A., Premont, R. T., and Lefkowitz, R. J. (1999) *J. Cell Biol.* 145, 927–932
12. Liu, F., Wan, Q., Pristupa, Z. B., Yu, X. M., Wang, Y. T., and Niznik, H. B. (2000) *Nature* 403, 274–280
13. Charpentier, S., Jarvie, K. R., Severynse, D. M., Caron, M. G., and Tiberi, M. (1997) *Neuron* 19, 233–243
14. Lefkowitz, R. J., Cotecchia, S., Samama, P., and Costa, T. (1993) *Annu. Rev. Pharmacol. Toxicol.* 33, 99–125
15. Ariano, M. A., Wang, J., Noblett, K. L., Larson, E. R., and Sibley, D. R. (1997) *J. Biol. Chem.* 272, 31882–31890
16. Lin, P. J., and Neve, R. L. (1997) *J. Biol. Chem.* 272, 30299–30306
17. Zhang, J., Barak, L. S., Anbergh, P. H., Laporte, S. A., Caron, M. G., and Ferguson, S. S. G. (1999) *J. Biol. Chem.* 274, 10999–11006
18. Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., and Caron, M. G. (1998) *Physiol. Rev.* 78, 189–225
19. Cai, G., Gurdal, H., Smith, C., Wang, H. Y., and Friedman, E. (1999) *Trends Pharmacol. Sci.* 20, 75–80
20. Tiberi, M., and Caron, M. G. (1994) *J. Biol. Chem.* 269, 27193–27200
21. Sheng, M., and Souzerai, S. (1998) *J. Cell Biol.* 140, 1457–1471
22. Tagawa, T., Sugamori, K. S., Scheideler, M. A., Vernier, P., and Niznik, H. B. (1998) *Nature* 394, 927–932
23. Charpentier, S., Jarvie, K. R., Severynse, D. M., Caron, M. G., and Tiberi, M. (1997) *Neuron* 19, 233–243
24. Niznik, H. B., Liu, F., Sugamori, K. S., Cardinaud, B., and Vernier, P. (1998) *J. Biol. Chem.* 273, 425–432
25. Namba, T., Sugimoto, Y., Negishi, M., Irie, A., Ushikubi, F., Kakizuka, A., He, S., Ichikawa, A., and Narumiya, A. (1993) *Nature* 365, 166–170
26. Hall, R. A., Premont, R. T., and Lefkowitz, R. J. (1999) *J. Cell Biol.* 145, 927–932
27. Liu, F., Wan, Q., Pristupa, Z. B., Yu, X. M., Wang, Y. T., and Niznik, H. B. (2000) *Nature* 403, 274–280
28. Demchyshyn, L. L., Sugamori, K. S., Lee, F. J. S., Hamadanizadeh, S. A., and Niznik, H. B. (1995) *J. Biol. Chem.* 270, 4005–4012
29. Sugamori, K. S., Scheider, M. A., Vernier, P., and Niznik, H. B. (1998) *J. Neurochem.* 71, 2593–2599
30. Kozell, L. B., and Neve, R. L. (1997) *J. Biol. Chem.* 272, 1137–1149
31. Niznik, H. B., McGill, J. M., Guibert, B., Sugamori, K. S., Liu, F., Niznik, H. B., and Vernier, P. (2000) in *Handbook of Experimental Pharmacology in the CNS* (Di Chiara, G., ed) Springer-Verlag, New York, in press
32. Drago, J., Padungchaichot, P., Accili, D., and Fuchs, S. (1998) *Proc. Natl. Acad. Sci. U. S. A.*, in press
33. Sibley, D. R. (1999) *Annu. Rev. Pharmacol. Toxicol.* 39, 313–341
34. Waddington, J. L., Deveney, A. M., Clifford, J. J., Tieghi, O., Croke, D. T., Sibley, D. R., and Drago, J. (1998) *Adv. Pharmacol.* 42, 514–517
35. Bergson, C., Mrzeljak, L., Smiley, J. F., Pappo, M., Levenson, R., and Goldman-Rakic, P. S. (1995) *J. Neurosci.* 15, 7821–7836
36. Ariano, M. A., Wang, S., Noblett, K. L., Larson, E. R., and Sibley, D. R. (1997) *Brain Res.* 746, 141–150
37. Smiley, J. F., Levey, A. I., Ciliax, B. J., and Goldman-Rakic, P. S. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 5720–5724
38. Sobell, J. L., Lind, T. J., Sigurdson, D. C., Zald, D. H., Snitz, B. E., Greve, W. M., Heston, L. L., and Sommer, S. S. (1995) *Hum. Mol. Genet.* 4, 507–514
39. Cravchik, A., and Gejman, P. V. (1999) *Pharmacogenetics* 9, 199–206
40. Feng, J., Sobell, J. L., Heston, L. L., Cook, E. H., Goldman, D., and Sommer, S. S. (1998) *Am. J. Med. Genet.* 81, 178–183