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# Molecular Cloning and Expression of a Dopamine D2 Receptor from Human Retina

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**SUMMARY**

Based on the sequence of a dopamine D2 receptor cloned from rat brain, we prepared a series of oligodeoxynucleotide probes. A mixture of these probes hybridized with a 2.6-kilobase species of mRNA extracted from several rat tissues including retina and, using *in situ* hybridization of these probes to cryostat sections of rat retina, they densely label the inner nuclear and outer plexiform layers. Labeling was also observed in the inner plexiform and ganglion cell layers. No hybridization was observed to the photoreceptor layers. A similar pattern of labeling was observed in monkey retina, indicating that the probes also hybridize with a homologous primate mRNA. The probes were used to screen a λgt10 library of human retina. A 2.5-kilobase clone was isolated, which encodes a protein that differs from the rat brain protein by 18 amino acids. The 5′ and 3′ untranslated regions of the human retinal cDNA were also strongly homologous with the rat brain cDNA. The clone was subcloned into the pCD-PS expression vector and transfected into COS-7 cells. The transfected cells bound [$^3$H]-raclopride with a pharmacology expected of dopamine D2 receptors. These data indicate that D2 receptors expressed in the inner retina and outer plexiform layer have genetic identity with those expressed by brain and that the human and rat D2 receptors are derived from highly related genes.

Dopamine is the major catecholamine present in retina, where its synthesis and release are stimulated by light (1). Dopamine is synthesized by amacrine cells in the inner retina, and these cells innervate other amacrine cells and horizontal cells in the outer plexiform layer (2). The physiological effects of dopamine in retina include a closing of gap junctions between horizontal cells (3), decreases in their responsiveness (4), shifts in the center-surround balance of the receptive fields of ganglion cells (5, 6), an involvement in the shedding (7), and light-adaptive movements of photoreceptors (8). As in brain (9), retinal dopamine receptors have been divided into two subtypes based on their pharmacological and functional properties (10). Dopamine D1 receptors stimulate adenylate cyclase by coupling with the G protein $G_s$, and D2 receptors inhibit adenylate cyclase by coupling with the G protein $G_i$ (10). Various drugs discriminate between these receptors; for example, SCH23396 has higher affinity for D1 receptors, whereas substituted benzamides such as sulpiride and raclopride have higher affinity for D2 receptors (10, 11). Physiological responses in the retina are mediated by both D1 and D2 receptors. D1 receptors mediate responses of horizontal cells (4), whereas D2 receptors are located on photoreceptors (12, 13) and mediate their light adaptive movements (8). D2 receptors also inhibit the release of dopamine from amacrine cells (14).

A dopamine D2 receptor from rat brain was recently cloned, sequenced, and expressed (15). Using Northern blot analysis, mRNA encoding this receptor has been observed in various brain regions known to contain D2 receptors (15). Using *in situ* hybridization histochemistry, mRNA encoding this receptor has recently been shown to be expressed by cells that are both pre- and postsynaptically located with respect to three major ascending dopaminergic pathways, the nigrostriatal, mesolimbic, and mesocortical pathways (16). To examine whether this gene accounts for the known distribution of D2 receptors within the retina, we localized mRNA within rat retina using oligodeoxynucleotide probes directed to the rat sequence. Because these probes were also able to detect mRNA in monkey retina, indicating homology within the probes to primate mRNA, we used these probes to screen a human retinal cDNA library. A human retinal cDNA was cloned, sequenced, and expressed in mammalian cells.

## Materials and Methods

**Preparation of oligodeoxynucleotides.** Five oligodeoxynucleotide probes were prepared on an Applied Biosystems automated DNA synthesizer (courtesy of P. Kelly, *Laboratory of Molecular Biology, National Institute of Neurological Disorders and Stroke*) and purified by preparative gel electrophoresis. The sequences of the probes were

**ABBREVIATIONS:** G protein, GTP-binding protein; Kb, kilobase.

complementary to bases 4–51 (S1: 5′-GTT CTG CCT CTC CAG ATC GTC ATC GTA CCA GGA CAG GTT CAG TGG ATC-3′), bases 954–1001 (S2: 5′-CAG GAC CTT GTT CTG CTG CTC CAG CTC GTG CAC GCG CTC GAT GAA GCT-3′), bases 1198–1245 (S3: 5′-GCA GTG CAA GAT CTT CAT GAA GGC CTT GCG GAA CTC GAT GTT GAA GGT-3′), bases 217–267 (TM2: 5′-CGG CAT TAC CAG TGT GGC CAC CAG AAG ATC AGC CAC AGC AAG GCT GAC TAT-3′), and bases 352–400 (TM3: 5′-GTA CCT GTC AAT GCT GAT GGC ACA CAG GTT CAG GAT GCT TGC TGT GCA-3′). Probes S1, S2, and S3 are complementary to regions of the mRNA that encode the N-terminal, third cytoplasmic loop, and C-terminal of the D2 receptor, respectively. Probes TM2 and TM3 are complementary to regions of the mRNA that encode the second and third transmembrane domains of the receptor. Three probes were prepared that are complementary to mRNA encoding the $\alpha$ subunit of mouse rod transducin (17). The sequences of the probes were complementary to mRNA encoding amino acids 100–115 (5′-CAT TGT GCC TTC CTC AAT AGT ATC TGC CAT GTG CAT GAG CGG CCG GGC-3′), amino acids 290–305 (5′-CTC CAG GAA CTG CAC TTT GAT GTA GTT GCC GGC ATC CTC GTA AGT GTT-3′), and amino acids 164–179 (5′-ACC AGT GGT TTT GAC CAC AGA ACG CAA CAC GTC CTG CTC AGT GGG CAC-3′). The probes were labeled by tailing the 3′ ends with terminal deoxynucleotide transferase (BRL), using $\alpha$-$^{35}$S-dATP as a substrate for those to be used for *in situ* hybridizations and $\alpha$-$^{32}$P]dATP for those to be used for Northern blots. For library screening, the probes were labeled by phosphorylating the 5′ ends with polynucleotide kinase (USB) using [$\gamma$-$^{32}$P]ATP as a substrate.

**Northern blot analysis.** Retinas and brain regions were dissected from male Sprague-Dawley rats and frozen on powdered dry ice. RNA was extracted using a guanidinium isothiocyanate method (18). Fifteen micrograms of total RNA were run on each lane of a formaldehyde-containing agarose gel and were transferred to Genescreen (NEN). Blots were prehybridized for >2 hr in hybridization buffer (4× SSPE, 50% formamide, 1× Denhardt's, 250 $\mu$g/ml sheared salmon sperm DNA) and hybridized at 37° for >18 hr with $9 \times 10^6$ dpm of a mixture of the S1, S2, and S3 probes. Denhardt's solution is 0.02% Ficoll, 0.02% bovine serum albumin, and 0.02% polyvinylpyrrolidone and 1× SSPE is (in mM) NaCl, 180; $NaH_2PO_4$, 10; and EDTA, 1. Blots were washed four times for 15 min at 55° and twice for 30 min at room temperature in 1× SSPE and exposed to X-ray film for 4 days with intensification screens. Sizes of bands were estimated by comparison with standards (BRL).

***In situ* hybridization histochemistry.** Eyes were removed from male Sprague-Dawley rats and frozen whole on dry ice. Retinas from the eyes of Rhesus monkeys (courtesy of Dr. G. Chader, National Eye Institute) were dissected, mounted flat in embedding medium, and frozen on dry ice. Twelve-micrometer cryostat sections were prepared (perpendicular to the surface of the retina in each case) and thaw-mounted on gelatin-coated slides. A mixture of the S1, S2, and S3 or transducin $\alpha$ probes ($9 \times 10^6$ dpm) was applied to each section in 50 $\mu$l of hybridization buffer (4× SSC, 50% formamide, 1× Denhardt's, 250 $\mu$g/ml sheared salmon sperm DNA, 100 mM dithiothreitol, 10% dextran sulfate). Sections were incubated for >18 hr in a humid chamber at 37°. Sections were washed four times for 15 min at 55° and twice for 30 min at room temperature in 1× SSC (in mM: NaCl, 150; Na citrate, 15).

**Screening of the human retinal library.** The clone λ16A was isolated from a human retinal λgt10 cDNA library obtained from Dr. J. Nathans (Johns Hopkins University School of Medicine, Baltimore, MD). Approximately $1 \times 10^6$ plaques were lifted in duplicate onto Hybond-N nylon filters (Amersham). One set of filters was screened with a mixture of the S1, S2, and S3 deoxyoligonucleotide probes and the duplicate set of filters was screened with a mixture of the TM2 and TM3 probes. Hybridizations were carried out at 42° in 20% formamide (v/v), 1 M NaCl, 50 mM Tris·HCl (pH 7.5), 5× concentrated Denhardt's solution, 0.5% sodium dodecyl sulfate containing 100 $\mu$g of salmon sperm DNA/ml. The filters were washed twice for 15 min at room temperature and twice for 1 hr at 47° in 1× SSPE, 0.5% sodium dodecyl sulfate. Clones that hybridized with both mixtures of probes were plaque purified and rescreened with the S1 probe alone to identify those most likely to be full length.

**Sequence determination.** The 2.5-kb *Eco*RI insert of λ16A was subcloned into the *Eco*RI site of the Bluescript II KS+ plasmid (Stratagene). The nucleotide sequences of the cDNA were determined by the dideoxy chain termination method using synthetic 17-mers derived from the rat D2 receptor (15) and λ16A sequences as primers for double-stranded plasmid sequencing (19).

**Expression and transfection.** The 2.5-kb *Eco*RI insert of λ16A was subcloned into the pCD-PS expression vector (20) and plasmid DNA was purified by using two cesium chloride gradients. COS-7 cells were plated at a density of $1 \times 10^6$ cells/10-cm dish. One to 2 days after plating, cells were transfected with 16A-pCD-PS DNA using a calcium phosphate precipitation procedure (21).

**Radioligand binding.** Cells were scraped, 48–72 hr after transfection, into 10 ml of phosphate-buffered saline/10-cm dish and pelleted at $1000 \times g$. Cells were resuspended in 10 mM Tris, 1 mM EDTA, 5 mM $MgCl_2$, and homogenized with a Polytron (Brinkman). A total particulate fraction was prepared by centrifugation at $45{,}000 \times g$ for 15 min. Membranes were resuspended in binding buffer (in mM: Tris-HCl, 50; NaCl, 120; $MgCl_2$, 1; $CaCl_2$, 1; KCl, 5; ascorbic acid, 5.7; pH 7.4) with ~10 $\mu$g protein/tube, 0.4 ml/tube. Reactions were initiated by addition of 0.4 ml of tissue in binding buffer to 0.1 ml of drugs. For saturation experiments, increasing concentrations of [$^3$H]raclopride (NEN) and [$^3$H]SCH23396 (NEN) were incubated with membranes for 30 min at 30°. Nonspecific binding was defined with 1 $\mu$M chlorpromazine. Reactions were terminated by filtering membranes through Whatman GF/C filters using a cell harvester (Brandel). Data were analyzed by nonlinear regression on a VAX II computer using the program DATAPLOT (distributed by the National Technical Information Service). For saturation experiments, the data were fit to the equation $y = (ax^nk)/(1 + x^nk)$ where $y$ = ligand bound, $x$ = ligand concentration, $a$ = maximal number of binding sites, $n$ = Hill number, and $k = k_D$. For inhibition experiments, the data were fit to the equation $y = (100x^nk)/(1 + x^nk)$ where $y$ = percentage of inhibition, $x$ = concentration of inhibitor, and $k$ = inhibition constant.

## Results

**Distribution of retinal D2 receptor mRNA.** Because the N-terminal, C-terminal, and third loop of the dopamine D2 receptor diverge in sequence from all other G protein-coupled receptors that have been cloned to date, probes (S1, S2, and S3) that are complementary to a portion of these regions were used to localize mRNA to ensure specificity. As we have previously reported (16), the specificity of the probes was verified based on three criteria. First, when the three probes are used for *in situ* hybridization histochemistry in adjacent sections of rat brain, they label identical patterns of hybridization and the intensity of hybridization is additive when the probes are combined. Second, on Northern blots of various brain regions including striatum, cerebral cortex, hippocampus, and brainstem, these probes label mRNA with a size of 2.6 kb, a size that is similar to that observed with a long probe cloned from the rat brain cDNA (15). Third, the pattern of hybridization observed in retina with these probes is distinct from that observed for other probes that are chemically similar, i.e., those for tyrosine hydroxylase, rhodopsin, and transducin (22, 23). For direct comparisons, three probes that are complementary to transducin mRNA were used for parallel hybridizations (Fig. 1). Transducin $\alpha$ is known to be located exclusively in photoreceptors (17, 22, 23).

On a Northern blot of rat retina, a 2.6-kb mRNA was

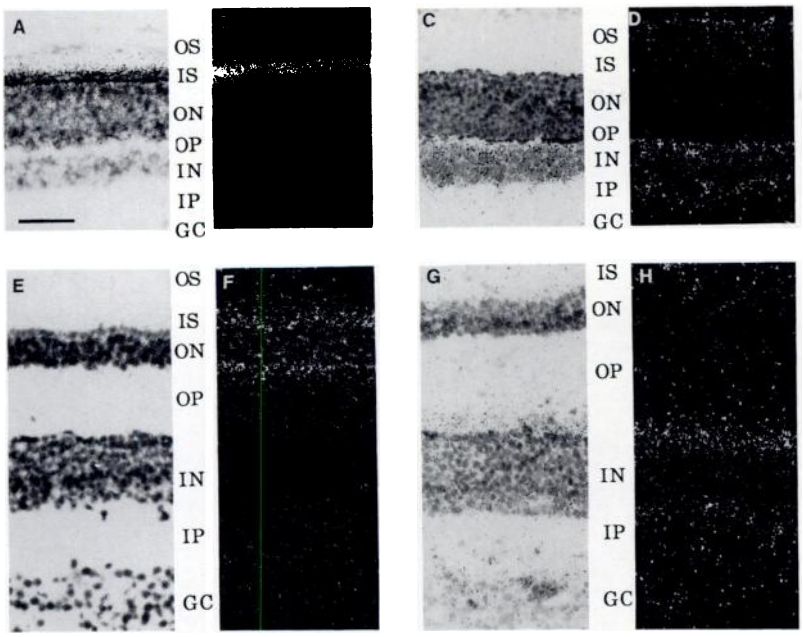


**Fig. 1.** Localization of D2 receptor (C, D, G, and H) and transducin $\alpha$ mRNA (A, B, E, and F) in rat (A–D) and monkey (E–H) retinas by *in situ* hybridization histochemistry. mRNA was autoradiographically localized by dipping the sections in emulsion (NTB3; Kodak; 1:1 with water) and exposing them for 2 weeks. Autoradiographic grains appear as black dots in the brightfield photomicrographs (A, C, E, and G) and as white dots in darkfield (B, D, F, and H). Cells were then counterstained with cresyl violet. The bar is 50 $\mu$m. *OS*, outer segment; *IS*, inner segment; *ON*, outer nuclear layer; *OP*, outer plexiform layer; *IN*, inner nuclear layer; *IP*, inner plexiform layer; *GC*, ganglion cell layer.

detected, and this mRNA was less abundant than that detected in striatum but more abundant than that detected in cerebral cortex, hippocampus, and brainstem (data not shown). As illustrated in Fig. 1, *in situ* hybridization histochemistry using sections of rat retina revealed hybridization of the D2 receptor probes to the inner nuclear and outer plexiform layers. Hybridization was also detected in a few cells in the ganglion cell layer (not shown). No significant hybridization was detected in the outer nuclear layer, inner segments, outer segments, or pigment epithelium [positive chemography of pigment epithelium with photographic emulsion makes detection of specific signal within those cells problematic (12, 22)]. A similar pattern of hybridization of the D2 receptor probes was detected in sections of monkey retina. Again, hybridization was detected in the inner nuclear and outer plexiform layers and no hybridization in the other layers. On the other hand, the transducin probes (a photoreceptor specific control) only hybridized to the inner segments and outer nuclear layer of both the monkey and rat retinas.

**Molecular cloning of a homologous cDNA from human retina.** Because the mixture of S1, S2, and S3 probes was able to detect a homologous mRNA that is present in primate retina, these probes were used to screen a human retinal λgt10 cDNA library lifted onto nylon filters. A duplicate set of lifts was screened with a mixture of the TM2 and TM3 probes. Although the latter probes may not be expected to be selective for dopamine receptors because the dopamine D2 receptor is homologous with all G protein-coupled receptors in the regions of these probes, they would be likely to detect the human homolog of the dopamine D2 receptor. Eight clones were positive with both sets of probes in the initial screens, and four of these clones hybridized with the N-terminal probe alone. Of these four, clone λ16A contained the largest cDNA insert (2.5 kb). This clone was subcloned into the Bluescript II KS+ vector for sequence analysis and the pCD-PS vector for mammalian expression.

**Comparison of the human and rat cDNAs.** The nucleic acid sequence of clone 16A is shown in Fig. 2. Comparison of the 5′ untranslated sequences of the human and rat cDNAs indicates a high degree of conservation, showing 91% identity. The coding regions of the two cDNAs are also highly conserved in sequence, showing 90% identity at the nucleic acid level. Comparison of the deduced amino acid sequence of the two proteins indicates the presence of 18 amino acid substitutions. Fourteen of these substitutions are conservative and nine are located in the third cytoplasmic loop. The human receptor is one amino acid shorter, lacking an isoleucine in the third cytoplasmic loop. Four amino acid substitutions are present in the hydrophobic (hypothetically transmembrane) domains of the proteins. Two of these substitutions are conservative.

**Mammalian expression of the human cDNA.** Before transfection, COS-7 cells do not express binding sites for [$^3$H] raclopride or [$^3$H]SCH23396 that are displaced by chlorpromazine. After transfection with 16A-pCD-PS DNA, [$^3$H]raclopride bound to COS-7 cells with high affinity, $k_D$ = 3.2 ± 0.4 nM, and a maximum number of binding sites of 800 ± 110 fmol/mg of protein. [$^3$H]SCH23396 did not show significant displaceable binding to transfected COS-7 cells in concentrations up to 5 nM. Above these concentrations, significant binding was detected in transfected cells, but the $k_D$ and $B_{max}$ values could not be determined because of the very high levels of nonspecific binding observed at these ligand concentrations. As shown in Fig. 3, several dopaminergic drugs competed with high affinity for the binding sites in transfected cells labeled by [$^3$H]raclopride. The rank order of potency was chlorpromazine > (−)-sulpiride > apomorphine > (+)-sulpiride > dopamine. All of the ligands bound with mass action, not displaying Hill numbers that significantly differed from 1.

## Discussion

Within the inner retina and outer plexiform layer there is good agreement between the distribution of dopamine D2 receptor mRNA and ligand binding. The regions with the highest concentrations of D2 ligand binding are the inner nuclear and outer plexiform layers (12, 13). The mRNA distribution reported here directly parallels this distribution. One cell type located in the inner nuclear layer, which is physiologically

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      -168 GAATTCCGGCCTCCCGGCCGAGGAGAGTGGCGGCCCCGGACGGCTGCCGGAGGGGCGGCCGCGCGTGGATGCGGCGGG
AGCTGGAAGCCTCAAGCAGCCGGCGCCGTCTCTGCCCCGGGGCGCCCTATGGCTTGAAGAGCCTGGCCACCCAGTGGCTCCACCGCCCTG
-90                         10                        20                  Glu       30
MetAspProLeuAsnLeuSerTrpTyrAspAspAspLeuGluArgGlnAsnTrpSerArgProPheAsnGlySerAspGlyLysAlaAsp
ATGGATCCACTGAATCTGTCCTGGTATGATGATGATCTGGAGAGGCAGAACTGGAGCCGGCCCTTCAACGGGTCAGACGGGAAGGCGGAC
0                       Met40                 PheIle      50                            60
ArgProHisTyrAsnTyrTyrAlaThrLeuLeuThrLeuLeuIleAlaValIleValPheGlyAsnValLeuValCysMetAlaValSer
AGACCCCACTACAACTACTATGCCACACTGCTCACCCTGCTCATCGCTGTCATCGTCTTCGGCAACGTGCTGGTGTGCATGGCTGTGTCC
90                          70                        80                            90
ArgGluLysAlaLeuGlnThrThrThrAsnTyrLeuIleValSerLeuAlaValAlaAspLeuLeuValAlaThrLeuValMetProTrp
CGCGAGAAGGCGCTGCAGACCACCACCAACTACCTGATCGTCAGCCTCGCAGTGGCCGACCTCCTCGTCGCCACACTGGTCATGCCCTGG
180                         100                       110                           120
ValValTyrLeuGluValValGlyGluTrpLysPheSerArgIleHisCysAspIlePheValThrLeuAspValMetMetCysThrAla
GTTGTCTACCTGGAGGTGGTAGGTGAGTGGAAATTCAGCAGGATTCACTGTGACATCTTCGTCACTCTGGACGTCATGATGTGCACGGCG
270                         130                       140                           150
SerIleLeuAsnLeuCysAlaIleSerIleAspArgTyrThrAlaValAlaMetProMetLeuTyrAsnThrArgTyrSerSerLysArg
AGCATCCTGAACTTGTGTGCCATCAGCATCGACAGGTACACAGCTGTGGCCATGCCCATGCTGTACAATACGCGCTACAGCTCCAAGCGC
360                 Ala     160                       170                 Thr       180
ArgValThrValMetIleSerIleValTrpValLeuSerPheThrIleSerCysProLeuLeuPheGlyLeuAsnAsnAlaAspGlnAsn
CGGGTCACCGTCATGATCTCCATCGTCTGGGTCCTGTCCTTCACCATCTCCTGCCCACTCCTCTTCGGACTCAATAACGCAGACCAGAAC
450                         190                       200                           210
GluCysIleIleAlaAsnProAlaPheValValTyrSerSerIleValSerPheTyrValProPheIleValThrLeuLeuValTyrIle
GAGTGCATCATTGCCAACCCGGCCTTCGTGGTCTACTCCTCCATCGTCTCCTTCTACGTGCCCTTCATTGTCACCCTGCTGGTCTACATC
540                 Lys     220                       230                 Asn   LysThr   240
LysIleTyrIleValLeuArgArgArgArgLysArgValAsnThrLysArgSerSerArgAlaPheArgAlaHisLeuArgAlaProLeu
AAGATCTACATTGTCCTCCGCAGACGCCGCAAGCGAGTCAACACCAAACGCAGCAGCCGAGCTTTCAGGGCCCACCTGAGGGCTCCACTA
630Asp                      250                       260                           270
LysGluAlaAlaArgArgAlaGlnGluLeuGluMetGluMetLeuSerSerThrSerProProGluArgThrArgTyrSerProIlePro
AAGGAGGCTGCCCGGCGAGCCCAGGAGCTGGAGATGGAGATGCTCTCCAGCACCAGCCCACCCGAGAGGACCCGGTACAGCCCCATCCCA
720                         280                     Asn290                          300
ProSerHisHisGlnLeuThrLeuProAspProSerHisHisGlyLeuHisSerThrProAspSerProAlaLysProGluLysAsnGly
CCCAGCCACCACCAGCTGACTCTCCCCGACCCGTCCCACCATGGTCTCCACAGCACTCCCGACAGCCCCGCCAAACCAGAGAAGAATGGG
810     *ValAsn   Arg       310Phe                  320                           330
HisAlaLysAsnHisProLysIleAlaLysIlePheGluIleGlnThrMetProAsnGlyLysThrArgThrSerLeuLysThrMetSer
CATGCCAAAGACCACCCCAAGATTGCCAAGATCTTTGAGATCCAGACCATGCCCAATGGCAAAACCCGGACCTCCCTCAAGACCATGAGC
900                         340                       350                           360
ArgArgLysLeuSerGlnGlnLysGluLysLysAlaThrGlnMetLeuAlaIleValLeuGlyValPheIleIleCysTrpLeuProPhe
CGTAGGAAGCTCTCCCAGCAGAAGGAGAAGAAAGCCACTCAGATGCTCGCCATTGTTCTCGGCGTGTTCATCATCTGCTGGCTGCCCTTC
990                         370                       380                           390
PheIleThrHisIleLeuAsnIleHisCysAspCysAsnIleProProValLeuTyrSerAlaPheThrTrpLeuGlyTyrValAsnSer
TTCATCACACACATCCTGAACATACACTGTGACTGCAACATCCCGCCTGTCCTGTACAGCGCCTTCACGTGGCTGGGCTATGTCAACAGC
1080                        400                     Met       414
AlaValAsnProIleIleTyrThrThrPheAsnIleGluPheArgLysAlaPheLeuLysIleLeuHisCysEnd
GCCGTGAACCCCATCATCTACACCACCTTCAACATTGAGTTCCGCAAGGCCTTCCTGAAGATCCTCCACTGCTGACTCTGCTGCCTGCCC
1170
GCACAGCAGCCTGCTTTCCACCTCCCTGCCCAGGCCGGTCCAGCCGTCACCCTTGCGAACCGTGAGCAGGAAGGCCTGGGTGGATCGGCC
TCCTCTTCACCCCGGCAGCCCTGCAGTGTTCGCTTGGCTCCATGCTCCTCACTGCCCCGCACACCCTCACTCTGCCAGGGCAGTGCTAGTG
AGCTGGGCATGGTACCAGCCCTGGGGCTCCCCCCAGCTCAGGGGCAGCTCATAGAGTCCCCCCTCCCACCTCCAGTCCCCCTATCCTTGG
CACCAAAGATCGAGCCGCCTTCCTTGACCTTCCTCTGGGCTCTAGGGTTGCTGGAGCCTGAGTCAGGGCCCAGAGGCTGAGTTTTCTCTT
TGTGGGGCTTGGCGTGGAGCAGGCGGTGGGGAGAGATGGACAGTTCACACCCTGCAAGGCCCACAGGAGGCAAGCAAGCTCTCTTGCCGA
GGAGCCAGGCAACTTCAGTCCTGGGAGACCCATGTAAATACCAGACTGCAGGTTGGACCCCAGAGATTCCCAAGCCGAAAAACCTTAGCT
CCCTCCCGGCACCCCGATGTGACCTCTACTTTCCAGGCTAGTCCGACCCACCTCACCCCGTTACAGCTCCCCAAGTGGTTTCCACATGCT
CTGAGAAGAGGAGCCCTCATCTTGAAGGGCCAGGAGGGTCTATGGGGAGAGGAACTCCTTGCCTAGCCCACCCTGCTGCCTTCTGACGGC
CCTGCAATGTATCCCTTCTCACAGCACATGCTGGCCAGCCTGGGGCCTGGCATGGTAGGCTCAGTCCCTGTAACTCTATCTGGGCCTGGG
CTAGGGTACATCAGAGGTTCTTTGAGGGACTGCCTCTGCCACACTCTGACAGCAAAACCACTTTCCTTTTCTATTCCTTCTGGCCTTTCC
TCTCTCCTGTTTCCCTTCGCTTCCACTGCCTCTGCCTTAGAGGACCCACGGCTAAGAGGCTGCTGAAAACCATCTGGCCTGGCCTGGCCC
TGCCCTGAGGAAGGAGGGGAAGCTGCAGCTTGGGAGAGCCCCTGGGGCCTAGACTCTGTAACATCACTATCCATGCACCAAACTAATAAA
ACTTTGACGAGTCACCTTCCCGGAATTC 2367
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**Fig. 2.** Sequence of human retinal dopamine D2 receptor cDNA. Sequence was determined by the dideoxynucleotide chain termination method. *Numbers of nucleotides* are indicated *below* the sequences in *italics*, and the *amino acids* are *numbered above*. Where the human and rat amino acids differ, the human amino acid is *underlined*, and the rat substitution is indicated *above*. The *asterisk* indicates the position of an extra isoleucine in the rat sequence.

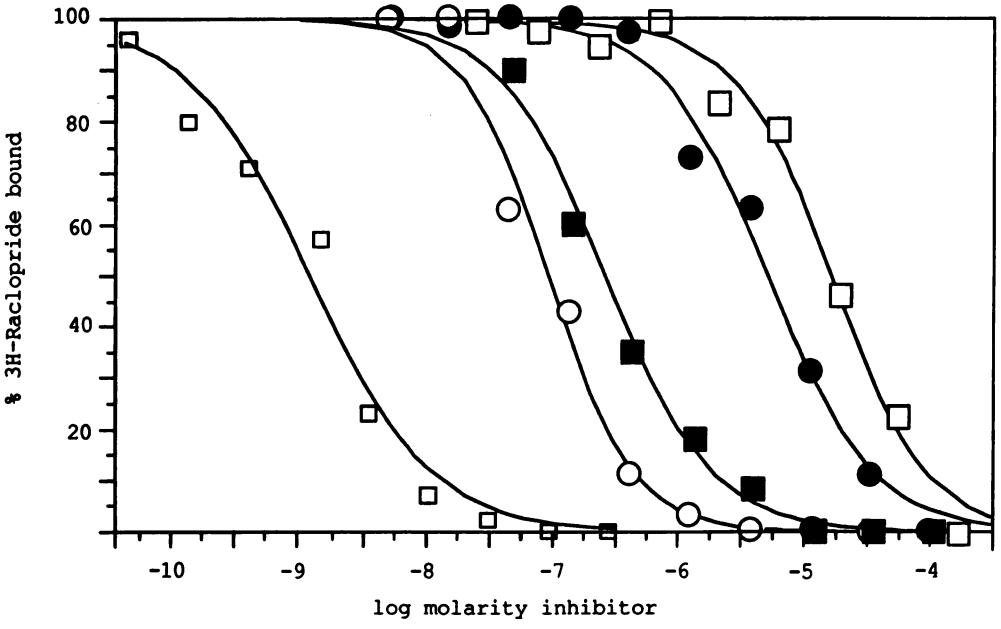


**Fig. 3.** Pharmacological analysis of sites labeled by [$^3$H]raclopride in COS-7 cells. Sites were labeled with 0.7 nM [$^3$H]raclopride and nonspecific binding was defined with 1 $\mu$M chlorpromazine. Data illustrate a single experiment; similar results were obtained in two or three independent experiments. *Lines* indicate best fits of the data to the function $y = (100 - 100\ x^n)/k/(1 + x^n k)$, where $x$ = inhibitor concentration, $n$ = Hill number, $k$ = inhibition constant ($\mu$M), and $y$ = percentage of [$^3$H]raclopride bound. These parameters for each of the inhibitors are as follows: chlorpromazine □, $k = 0.00150 \pm 0.13$, $n = 0.9 \pm 0.1$; (−)-sulpiride (○), $k = 0.046 \pm 0.02$, $n = 1.2 \pm 0.2$; apomorphine (■), $k = 0.25 \pm 0.03$, $n = 1.0 \pm 0.1$; (+)-sulpiride (●), $k = 5.45 \pm 1.08$, $n = 1.0 \pm 0.1$; dopamine (□), $k = 24.8 \pm 8.17$, $n = 1.1 \pm 0.1$.

known to have dopamine receptors, is the dopaminergic amacrine cell, where D2 receptors inhibit the release of dopamine (14). Because D2 receptor mRNA is widely distributed throughout the inner nuclear layer and dopaminergic cells are very rare, our data indicate that other cells in addition to dopamine neurons must express this mRNA. Processes from dopaminergic neurons are widely distributed in the inner retina and outer plexiform layer (2, 24), consistent with the majority of mRNA observed here being made by cells that are postsynaptic with respect to dopaminergic neurons. We have not morphologically defined the phenotype of the few cells located in the ganglion cell layer that express high concentrations of D2 receptor mRNA. One possibility, which is consistent with their relative sparseness, is that these cells represent displaced amacrine cells (24).

Within the outer retina there is a marked difference in the distribution of D2 receptor mRNA and ligand binding. Binding data have indicated that a high concentration of dopamine D2 receptors is present in photoreceptors, being present in the outer nuclear layer as well as inner and outer segments (12, 13). In addition to the binding data, physiological data collected from various species have indicated a physiological role for dopamine receptors on photoreceptors (7, 8). In our experiments with both monkey and rat retina, we have failed to detect D2 receptor mRNA within photoreceptors. The most likely explanation for this observation is that photoreceptors express dopamine D2 receptors that are encoded by a gene different from the one we have examined. Alternatively, photoreceptors may have a very different ratio of mRNA to protein than do other cells in retina and brain.

Evidence for a heterogeneity of dopamine D2 receptors was recently presented based on binding of [$^3$H]spiperone to human retina (25). These data indicate that the substituted benzamide sulpiride discriminates among sites that have identical affinities for [$^3$H]spiperone. The pharmacology of the receptor that we have cloned is like those that have the highest affinity for sulpiride. It is tempting to speculate that another D2 receptor subtype is yet to be cloned, which may be present in photoreceptors and may have a lower affinity for substituted benzamides.

The human and rat dopamine D2 receptors are encoded by highly related genes. Because the encoded proteins have very few amino acid substitutions within their proposed transmembrane domains, one would predict that the two receptors would be highly related pharmacologically. This prediction is based on a hypothetical analogy to the $\beta$-adrenergic receptor. $\beta$-Receptors are structurally and functionally related to dopamine D2 receptors, and the transmembrane domains are largely responsible for their pharmacological properties (26). The predicted pharmacological similarity between the rat and human receptors is borne out by the expression data. The binding sites expressed in COS-7 cells by the human cDNA have many similarities to sites observed on expression of the rat gene and sites observed in both human retina and rat brain. The fact that there are differences in the human and rat D2 receptors indicates the potential for subtle differences in their pharmacology.

The agonist pharmacologies of the cloned human and rat receptors do not agree with that observed for endogenously expressed receptors. For the receptors expressed by COS-7 cells, agonists show steep inhibition curves, consistent with the predominant presence of a low affinity form of the receptor. One explanation for this phenomenon is that in COS-7 cells the very high levels of receptor saturate the endogenous G proteins. In support of this possibility is the observation that, when muscarinic receptors are expressed at similar levels in COS-7 cells, they show similarly steep inhibition curves, while these receptors are still able to functionally couple with G proteins to stimulate inositol phosphate metabolism (27). On the other hand, when these receptors are expressed at lower levels in A9 L cells, agonists show broad inhibition curves (28). In order to evaluate the pharmacological properties of cloned D2 receptors with respect to agonists, it will be essential to express the receptors at levels that more closely approximate those found endogenously. It will also be necessary to express the receptors in cells that express the G proteins and functional pathways that dopamine receptors normally activate.

**References**


1. Iuvone, P. M., C. L. Galli, C. K. Garrison-Gund, and N. H. Neff. Light stimulates tyrosine hydroxylase activity and dopamine synthesis in retinal amacrine neurons. *Science* (*Wash. D. C.*) **202:**901–902 (1978).
2. Ehinger, B. Neurotransmitter systems in the retina. *Retina* **2:**305–321 (1982).
3. Lasater, E. M., and J. E. Dowling. Dopamine decreases conductance of the electrical junctions between cultured retinal horizontal cells. *Proc. Natl. Acad. Sci. USA* **82:**3025–3029 (1980).
4. Mangel, S.C., and J.E. Dowling. Responsiveness and receptive field size of carp horizontal cells are reduced by prolonged darkness and dopamine. *Science* (*Wash. D. C.*) **229:**1107–1108 (1985).
5. Jensen, J. R., and N. W. Daw. Effects of dopamine antagonists on receptive fields of brisk cells and directionally selective cells in the rabbit retina. *J. Neurosci.* **4:**2972–2985 (1984).
6. Thier, P., and V. Alder. Action of iontophoretically applied dopamine on cat retinal ganglion cells. *Brain Res.* **292:**109–121 (1984).
7. Reme, C. E., A. Wirtz-Justice, and M. Daprada. Retinal rhythms in photoreceptors and dopamine synthetic rate: the effect of a monoamine oxidase inhibitor. *Trans Opthalmol. Soc. UK* **103:**405–410 (1983).
8. Dearry, A., and B. Burnside. Dopamine inhibits forskolin- and 3-isobutylmethylxanthine-induced dark-adaptive retinomotor movements in isolated teleost retina. *J. Neurochem.* **44:**1753–1763 (1985).
9. Kebabian, J. W., and D. B. Calne. Multiple receptors for dopamine. *Nature* (*Lond.*) **277:**93–96 (1980).
10. Qu, Z. X., R. Fertel, N. H. Neff, and M. Hadjiconstantinou. Pharmacological characterization of rat retinal dopamine receptors. *J. Pharmacol. Exp. Ther.* **248:** 621–625 (1989).
11. Hall, H., and I. Wadel. Comparisons between the *in vitro* binding of two substituted benzamides and two butyrophenones to dopamine-D2 receptors in the rat striatum. *Acta Pharmacol. Toxicol.* **58:**368–373 (1986).
12. Brann, M. R., and W. S. Young III. Dopamine receptors are located on rods in bovine retina. *Neurosci. Lett.* **69:**221–226 (1986).
13. Zarbin, M. A., J. K. Wamsley, J. M. Palacios, and M. J. Kuhar. Autoradiographic localization of high affinity GABA, benzodiazepine, dopaminergic, adrenergic and muscarinic cholinergic receptors in the rat, monkey and human retina. *Brain Res.* **374:**75–92 (1986).
14. Dubocovich, M. Modulation of [$^3$H]dopamine release from rabbit retina. *Fed. Proc.* **43:**2714–2718 (1984).
15. Bunzow, J. R., H. H. M. Van Tol, D. K. Grandy, P. Albert, J. Salon, M. Christie, C. A. Machida, K. A. Neve, and O. Civelli. Cloning and expression of a rat D2 dopamine receptor cDNA. *Nature* (*Lond.*) **336:**783–787 (1988).
16. Weiner, D. M., and M. R. Brann. The distribution of a dopamine D2 receptor mRNA in rat brain. *FEBS Lett.* **253:**207–213 (1989).
17. Raport, C. J., B. Deve, and J. B. Hurley. Characterization of the mouse rod transducin $\alpha$ subunit gene. J. Biol Chem. **264:**7122–7128 (1989).
18. Chomczynski, P., and N. Sacchi. Single-step method of RNA isolation by acid guanidinium thiocyanate. *Anal. Biochem.* **162:**156–159 (1987).
19. Kraft, R. J., K. S. Tardoff, K. S. Krauter, and L. A. Leinwand. Using miniprep plasmid DNA for sequencing double-stranded templates with Sequenase. *Biotechniqes* **6:**544–546 (1988).
20. Bonner, T. I., A. C. Young, M. R. Brann, and N. J. Buckley. Cloning and expression of the human and rat m5 muscarinic receptor genes. *Neuron* **1:**403–410 (1988).
21. Chen, C., and H. Okayama. High-efficiency transformation of mammalian cells by plasmid DNA. *Mol. Cell. Biol.* **7:**2745–2752 (1987).
22. Brann, M. R., and W. S. Young III. Localization and quantitation of opsin and transducin mRNAs in bovine retina by *in situ* hybridization histochemistry. *FEBS Lett.* **200:**275–278 (1986).
23. Brann, M. R., and L. V. Cohen. Diurnal expression of transducin mRNA and translocation of transducin in rods of rat retina. *Science* (*Wash. D. C.*) **235:**585–587 (1987).

24. Ballesta, J., G. Tereughi, J. Thibault, and J. M. Polak. Putative dopamine-containing cells in the retina of seven species domonstrated by tyrosine hydroxylase immunocytochemistry. *Neuroscience* **12**:1147–1156 (1984).
25. McGonigle, P., M. B. Wax, and P. B. Molinoff. Characterization of binding sites for $^3$H-spiroperidol in human retina. *Invest. Ophthalmol. & Visual Sci.* **29**:687–694 (1988).
26. Dixon, R. A. F., I. S. Sigal, E. Rands, R. B. Register, M. R. Candelore, A. D. Blake, and C. D. Strader. Ligand binding to the $\beta$-adrenergic receptor involves its rhodopsin-like core. *Nature* (*Lond.*) **326**:73–77 (1987).
27. Wess, J., M. R. Brann, and T. I. Bonner. Identification of a small intracellular region of the muscarinic m3 receptor as a determinant of selective coupling to PI turnover. *FEBS Lett.*, in press.
28. Brann, M. R., N. J. Buckley, S. V. P. Jones, and T. I. Bonner. Expression of a cloned muscarinic receptor in A9 L cells. *Mol. Pharmacol.* **32**:450–455 (1987).

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