New dopamine receptor, D2\textsubscript{Longer}, with unique TG splice site, in human brain\(^1\)

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Abstract

Brain dopamine receptor agonists alleviate the signs of Parkinson’s disease, while dopamine receptor antagonists alleviate hallucinations and delusions in psychosis. The dopamine type 2 receptor (or D2) is blocked by antipsychotic drugs, including even the “atypical” drugs such as clozapine or remoxipride, in direct relation to their clinical potencies. Compared to the long form of the D2 receptor (D2\textsubscript{Long}), the short form (D2\textsubscript{Short}) may be three times more sensitive to benzamide antipsychotic drugs. Hence, it is essential to identify additional variants of dopamine receptors for which more selective antipsychotic drugs can be found. Although no family linkage has been found between the D2 receptor and schizophrenia, there can be brain region abnormalities in the RNA transcript expression of dopamine receptors. Therefore, in order to identify variant dopamine D2 receptors, we searched for mutations in the RNA transcripts for the dopamine D2 receptor in the striatum of post-mortem brains from individuals who died with psychosis, including schizophrenia. A new splice variant of the D2 receptor, D2\textsubscript{Longer}, with a unique TG splice site, was found in one control brain and in two psychotic brains.

1. Introduction

Dopamine receptor antagonists are effective antipsychotic drugs, alleviating hallucinations and delusions in neuropsychiatric disorders [40]. Improved therapy may be obtained by developing drugs that selectively target one of the several types of dopamine receptors [40]. For example, the dopamine type 2 receptor (or D2) is blocked by antipsychotic drugs, including even the “atypical” drugs such as clozapine or quetiapine, in direct relation to their clinical potency [39,41]. Compared to the long form of the D2 receptor (D2\textsubscript{Long}), the short form (D2\textsubscript{Short}) may be more sensitive to benzamide antipsychotic drugs [3,20,22]. Moreover, three other genomic variants of the D2 receptor [10,16], each with a single amino acid substitution, exhibit marked differences in their ability to inhibit cyclic AMP synthesis [6]. Hence, it is essential to identify additional variants of dopamine receptors for which more selective antipsychotic drugs can be found.

Although the dopamine D1 receptor is not the primary target of antipsychotic drugs [39], it indirectly affects the existence of the high-affinity state of the D2 receptor, an influence which is reduced in psychosis [42]. Although no association has been found between markers for either of these two receptors and schizophrenia [4,5,19,24,28,29,33,34,43] there can be brain region abnormalities in the RNA transcript expression of dopamine receptors, such as that for D3 and D4 dopamine receptors in schizophrenia [23]. Moreover, mutations [47,48] and editing [2,14,37,48] of RNA can occur independent of a normal genomic DNA. Therefore, in order to identify variant dopamine D2 receptors, we searched for mutations in the RNA transcripts for the dopamine D2 receptor in the striatum of post-mortem brains from individuals who died with psychosis, including schizophrenia. A new splice variant of the D2 receptor, D2\textsubscript{Longer}, with a unique TG splice site, was found in one control brain and in two psychotic brains.

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\(^1\) The sequence reported in this paper has been deposited in the GenBank database [accession no. AF176812].
2. Materials and methods

2.1. Human tissues

A summary of the case histories of the post-mortem human brains is given in Table 1. The frozen striata (caudate nucleus and putamen) and the frozen frontal cerebral cortices were used to search for variants in the RNA transcripts coding for the dopamine D2 receptor.

2.2. RNA and cDNA from post-mortem human tissues

The tissues were homogenized in TRIZOL® Reagent (Life Technologies, Burlington, Ontario, Gibco) and chloroform (0.2 ml per 1 ml of TRIZOL®). The RNA in the upper phase was precipitated from the aqueous phase by adding 0.5 ml of isopropanol per 1 ml of TRIZOL® Reagent. The samples were incubated at 15–30°C for 10 min and then centrifuged at 12,000 x g for 10 min at 4°C. After removing the supernate, the RNA pellet was washed once with 75% ethanol (1 ml per ml original TRIZOL® Reagent). The mixed sample was centrifuged at 7500 x g for 5 min at 4°C. The final RNA pellet was briefly air-dried, dissolved in 0.5% sodium dodecyl sulfate, and incubated for 10 min at 55–60°C. Although it has generally been assumed that mRNA degradation renders post-mortem human brain research unreliable, many studies have shown that mRNA transcripts are relatively stable in human post-mortem tissues [11,17,30,32]. cDNA was then prepared for each tissue, using 2 μg of total RNA, and using the procedure described in the Superscript™ Preamplification System for First Strand cDNA synthesis (Catalogue 18089-011, Gibco BRL products, Life Technologies). The cDNA was amplified by the polymerase chain reaction (PCR) procedure, using primers N25 and N1495 (see following sequences) for the D2 receptor, using a GeneAmp PCR System 9600 (Perkin-Elmer Cetus, Foster City, CA) thermocycler, and using Advantage®-HF (High Fidelity) DNA polymerase (Kit PT3139-1, Catalogue # K1909-1, CLON-TECH Laboratories, Palo Alto, CA).

The sequence of primer N25 was (5’) ACC GCC CTG ATG GAT CCA CTG AAT CTG TCC(3’), and the sequence of primer N1495 was (5’) TGA GGA GCA TGG AGCCAA GCG AAC ACT GC(3’). The manufacturer’s procedure for Advantage®-HF DNA polymerase was followed. Thus, to amplify the cDNA for the D2 receptor, each PCR tube received 0.8 μg of cDNA, 2.5 μl of High-Fidelity buffer (supplied by the manufacturer), 2.5 μl of 10 mM dNTPs (dATP, dTTP, dCTP, dGTP), 0.5 μl (0.5 μg) of primer N25, 0.5 μl (0.5 μg) of primer N1495, 0.5 μl of DNA polymerase (Advantage®-HF), and 17.5 μl water. A total of 35 thermocycles was done, each consisting of 15 s for denaturation at 94°C, 4 min for annealing and extension at 68°C, followed by a final period of 7 min for extension at 68°C. The PCR products were electrophoresed on agarose gels (1.5%) to confirm the MW of the expected bands (1.4 kb for D2). An aliquot of the PCR products was subcloned into the vector pCR® 2.1 (Invitrogen, San Diego, CA), using the Original TA Cloning® Kit (Invitrogen, Catalog no. K2000-01 and K2000-40). Colonies were picked for DNA minipreparations and sequencing.

2.3. PCR amplification of cDNA for D2_Longer

Each PCR tube received 9.5 μl of water, 5 μl of 5X GC cDNA PCR Reaction buffer (Kit number PT3091-1, CLON-TECH Laboratories), 0.5 μl of 10 mM dNTPs, 0.5 μl of 10 μM primer 863 (General Synthesis and Diagnostics, Toronto), 0.5 μl of 10 μM primer 253 (see following for primer sequences), 0.5 μl of Advantage®-GC cDNA polymerase Mix (50X) (Kit number PT3091-1, CLON-TECH Laboratories), 7.5 μl of GC-Melt™ (CLON-TECH Laboratories), 1 μl of cDNA from human tissue or from the cloned DNA of D2_Long or D2_Longer, making a total volume of 25 μl. The sequence of primer 253 was (5’ GTC

Table 1
Clinical summaries for post-mortem brain tissues

<table>
<thead>
<tr>
<th>Brain</th>
<th>Age</th>
<th>sex</th>
<th>P.M. delay</th>
<th>Death</th>
<th>Diagnosis; symptoms</th>
<th>Duration antipsychotic Rx of psychosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control tissues</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L 964</td>
<td>29</td>
<td>m</td>
<td>24 h</td>
<td>Cycle accident</td>
<td>Control</td>
<td>None</td>
</tr>
<tr>
<td>T 1294</td>
<td>41</td>
<td>m</td>
<td>4 h</td>
<td>Car accident</td>
<td>Control</td>
<td>None</td>
</tr>
<tr>
<td><strong>Psychosis tissues</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L 261</td>
<td>53</td>
<td>m</td>
<td>10 h</td>
<td>Cardiac infarct</td>
<td>Schizophrenia; voices</td>
<td>10 yr</td>
</tr>
<tr>
<td>T 263</td>
<td>73</td>
<td>m</td>
<td>7 h</td>
<td>Coma</td>
<td>Alzheimer; delusional</td>
<td>&gt; 7 yr</td>
</tr>
<tr>
<td>S 117</td>
<td>64</td>
<td>f</td>
<td>32 h</td>
<td>Pneumonia</td>
<td>Schizophrenia; delusions</td>
<td>34 yr</td>
</tr>
<tr>
<td>S 130</td>
<td>65</td>
<td>m</td>
<td>6 h</td>
<td>Cardiac infarct</td>
<td>Schizophrenia; incoherent</td>
<td>25 yr</td>
</tr>
<tr>
<td>S 132</td>
<td>83</td>
<td>m</td>
<td>48 h</td>
<td>Cardiac?</td>
<td>Schizophrenia; delusions</td>
<td>57 yr</td>
</tr>
</tbody>
</table>
AGC CTC GCA GTG GCC GAC CTC CTC G(3’), and the sequence of primer 863 was (5’)TGG GCT CGC CGG GCA GCC TC CTG CAC (3’), where the latter six bolded letters indicate the bases complementary to the six additional bases found in the dopamine D2L longer receptor (see Section 3). The mixture was preincubated at 94°C for 5 min (‘hot start’), followed by 40 thermocycles, each consisting of 30 s for denaturation at 94°C, 60 s for annealing at 75°C, and followed by a final period of 5 min for extension at 75°C. Because of the low levels of the D2L longer cDNA in the extracts from the human frontal cerebral cortex, an aliquot of 1 μl of the latter PCR products for these tissues was subjected to a further 25 thermocycles. The predicted size of the PCR product was 610 bp.

2.4. Measurement of inhibition constants for drugs

The competition between a drug and a [3H]ligand for binding at the dopamine D2 receptors was done as follows. Each incubation tube (12 × 75 mm glass) received, in the following order, 0.5 ml buffer (50 mM Tris–HCl, pH 7.4 at 20°C, 1 mM EDTA, 5 mM KCl, 1.5 mM CaCl2, 4 mM MgCl2 and 120 mM NaCl) (with or without an excess of S-sulpiride; see below), 0.5 ml [3H]ligand (final concentration of approximately 200 pM for [3H]spiperone [60–100 Ci/mmol; 2.2–3.7 TBq/mmol; Amersham Life Sciences, Oakville, Ontario, Canada] and 2 nM for [3H]raclopride [60–70 Ci/mmol; New England Nuclear, E.I. du Pont de Nemours, Boston, MA]), and 0.5 ml of a suspension of the disrupted COS or 293 cells. The tissue culture cells were collected and suspended at approximately 200 μg protein/ml. The cells were not washed or centrifuged. The cell suspension was homogenized for 5 s (Polytron, setting 5; Brinkmann Instruments, Westbury, NY) immediately before being used.

The tubes containing a total volume of 1.5 ml were incubated for 2 h at room temperature (20°C), after which the incubates were filtered, using a 12-well cell harvester (Titertek, Skatron, Lier, Norway) and buffer-presoaked glass fiber filter mats (No. 7034, Skatron, Sterling, VA). After filtering the incubate, the filter mat was rinsed with buffer for 15 s (7.5 ml buffer). The filters were pushed out and placed in scintillation minivials (Packard Instruments, Chicago, IL). The minivials received 4 ml each of scintillant (Ready Solve, Beckman, CA), and were monitored 6 h later for tritium in a Packard 4660 scintillation spectrometer at 55% efficiency. Nonspecific binding for dopamine D2 receptors was defined as that which occurred in the presence of 10 μM S-sulpiride.

The Kd values (dissociation constants) of [3H]spiperone and [3H]raclopride for the dopamine D2 receptors were measured separately. This was done using final concentrations of 10 to 2000 pM for [3H]spiperone, and 0.2 to 12 nM for [3H]raclopride, and using the same procedure outlined above for the competition-type experiments. The dissociation constants were derived by Scatchard analysis.

The inhibition constant, Ki, for each drug was then derived by the traditional relation, Ki = C∞/(1 + [C∞/Ki]), where C∞ is the concentration of drug which inhibits the binding of the [3H]ligand by 50%, where C∞ is the final concentration of the [3H]ligand, and where the Ki is the dissociation constant of the [3H]ligand directly determined as outlined above.

3. Results

The objective of this study was to identify variant dopamine D2 receptors. Because mutations and editing of RNA can occur independent of a normal genomic DNA [2,14,37,47,48], we searched for mutations in the RNA transcripts for the dopamine D2 receptor in the striata of post-mortem brains. Because the dopamine D2 receptor is the main target for antipsychotic drugs, we hypothesized that there may be mutations in the D2 receptor which conceivably might be found in psychotic brain tissue from...
**Fig. 2. Exon and intron sequence for the dopamine D2<sub>Longer</sub> receptor.** The six bases which are normally included in intron 5 of D2<sub>Longer</sub> (gtg and cag) are part of exon 6 in D2<sub>Longer</sub> (GTG and CAG).
Table 3
Inhibition constants ($K_i$) of drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>$K_i$ D2&lt;sub&gt;Long&lt;/sub&gt; COS cells</th>
<th>$K_i$ D2&lt;sub&gt;Longer&lt;/sub&gt; COS cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonists</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dopamine</td>
<td>2.2 ± 0.3 μM</td>
<td>2.4 ± 0.4 μM</td>
</tr>
<tr>
<td>Dopamine&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.9 ± 0.4 μM</td>
<td>5.8 ± 0.8 μM</td>
</tr>
<tr>
<td>Dopamine&lt;sup&gt;b&lt;/sup&gt; + G.N.</td>
<td>10 ± 4 μM</td>
<td>27 ± 8 μM</td>
</tr>
<tr>
<td>Serotonin</td>
<td>11 ± 3 μM</td>
<td>11 ± 3 μM</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>15 ± 5 μM</td>
<td>18 ± 5 μM</td>
</tr>
<tr>
<td>Antagonists</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clozapine</td>
<td>45 ± 12 nM</td>
<td>66 ± 15 nM</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>0.21 ± 0.1 nM</td>
<td>0.22 ± 0.1 nM</td>
</tr>
<tr>
<td>Loxapine</td>
<td>1.5 ± 0.5 nM</td>
<td>1.8 ± 0.4 nM</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>7.4 ± 2 nM</td>
<td>9.2 ± 0.9 nM</td>
</tr>
<tr>
<td>Periapine</td>
<td>220 ± 80 nM</td>
<td>190 ± 60 nM</td>
</tr>
<tr>
<td>Raclopride</td>
<td>2.2 ± 0.4 nM</td>
<td>1.7 ± 0.4 nM</td>
</tr>
<tr>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]Raclopride</td>
<td>1.9 ± 0.5 nM</td>
<td>1.8 ± 0.6 nM</td>
</tr>
<tr>
<td>Remoxipride</td>
<td>79 ± 12 nM</td>
<td>69 ± 15 nM</td>
</tr>
<tr>
<td>Risperidone</td>
<td>0.53 ± 0.2 nM</td>
<td>0.7 ± 0.3 nM</td>
</tr>
<tr>
<td>Sertindole</td>
<td>0.39 ± 0.1 nM</td>
<td>1 ± 0.4 nM</td>
</tr>
<tr>
<td>[&lt;sup&gt;3&lt;/sup&gt;H]Sertindole</td>
<td>0.076 ± 0.01 nM</td>
<td>0.12 ± 0.05 nM</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>0.78 ± 0.1 nM</td>
<td>0.52 ± 0.2 nM</td>
</tr>
<tr>
<td>Ziprasidone</td>
<td>2.6 ± 0.7 nM</td>
<td>3.3 ± 0.4 nM</td>
</tr>
</tbody>
</table>

<sup>a</sup> Using 2 nM [<sup>3</sup>H]raclopride (70 Ci/mmmol) (n = 3); average ± S.E.M.
<sup>b</sup> Using 200 pM [<sup>3</sup>H]spiperone (101 Ci/mmmol) and 293 cells (n = 5).

G.N.: In the presence of 200 μM guanilylimidodiphosphate (n = 5).

Individuals who have died with psychosis, despite the fact that no abnormal sequences have been found in the genomic DNA of dopamine D2 receptors in such individuals. Hence, we examined brain tissues from individuals who died with psychosis (four individuals who had schizophrenia, and one who had Alzheimer’s disease with delusions) and from two individuals who died suddenly of accidents (car accident and motorcycle accident) (Table 1).

After preparing the RNA and cDNA from each tissue (see Section 2), the full-length cDNA for the dopamine D2 receptor was amplified by the PCR, using primers N25 and N1495 (see Section 2).

A total of 219 transcripts of the RNA for the dopamine D2 receptor were examined in tissues from seven brains, as summarized in Table 2. The full-length cDNA sequences were examined for almost all of these 219 transcripts. The findings in Table 2 show that 79% of the transcripts were D2<sub>Long</sub>, while 18% of the transcripts were D2<sub>Short</sub>.

During the course of sequencing the 219 cDNA transcripts, we were surprised to discover five sequences for the D2 receptor which contained six extra bases (GTG CAG) located immediately before the start of exon 6, as shown in Figs. 1 and 2. This variant of the D2 receptor, which may be named D2<sub>Longer</sub>, is essentially a new splice...
variant of the D2 receptor sequence, because the sequence of GTG CAG is normally found in the intron (i.e., intron 5) preceding the normal start of exon 6.

An additional surprise was to discover that this new splice variant of the D2 receptor, $D_{2L}$, had a unique TG splice site, as shown in Fig. 2. The unusual nature of this TG splice site is considered in Section 4.

The transcript variant, $D_{2L}$, was found in three striata (schizophrenia S-130, Alzheimer T-638 [who was delusional], and control brain T-1292). This transcript variant was identical to that for $D_{2S}$, except that the splice site between intron 5 and exon 6 occurred six bases before the start of exon 6 in $D_{2L}$, as shown in Figs. 1 and 2. Because the new dopamine D2 receptor which would be synthesized by this variant mRNA would contain an extra two amino acids (compared to $D_{2L}$), the new variant was named $D_{2L}$.

Because it was possible that these extra two amino acids (valine and glutamine) could alter the binding of dopamine agonists and antagonists to the dopamine $D_{2L}$ receptor (in comparison to $D_{2S}$ or $D_{2L}$), it was necessary to examine this directly. Therefore, the cDNA for the dopamine $D_{2L}$ receptor was transfected into, and expressed in, both COS and 293 cells, and the affinities of the expressed dopamine $D_{2L}$ receptor for various drugs were obtained. Both the COS cells and the 293 cells expressed approximately 1.5 to 2 pg/mg of protein.

The data in Table 3 indicate that the inhibition constants of the dopamine $D_{2L}$ receptor for various agonists and antagonists were similar, but not always identical, to those for the dopamine $D_{2L}$ receptor. Parenthetically, it may be noted that the inhibition constant for dopamine is slightly higher when competing against $[^{3}H]$spiperone than when competing against $[^{3}H] $raclopride. This apparent dependence of the inhibition constant on the $[^{3}H]$ligand results from the higher solubility of the $[^{3}H]$ligand in the membrane, as extensively described elsewhere [see Ref. of Seeman and Van Tol, 1995, in Ref. [39]].

Moreover, the binding of dopamine to the dopamine $D_{2L}$ receptor was sensitive to guanilylimidodiphosphate, suggesting that a portion of the dopamine $D_{2L}$ receptors were in the functional high-affinity state for dopamine [40].

Despite the low prevalence of the dopamine $D_{2L}$ receptor in the tissues examined, it was possible to amplify this receptor selectively from cDNA prepared from post-mortem human striatum and frontal cortex tissues, using the PCR, and using primers 253 and 863. This is shown in Fig. 3, where 40 cycles of PCR were sufficient to amplify the $D_{2L}$ cDNA from the striatum, while an additional 25 cycles of PCR were required to amplify the $D_{2S}$ cDNA from the frontal cortex. The selectivity of the conditions for amplifying $D_{2L}$ are illustrated by the lack of amplification of the cloned $D_{2L}$ cDNA (Fig. 3), despite the 65 cycles of PCR.

### 4. Discussion

The new variant, $D_{2L}$, was found in brain striata from both control and psychotic individuals (Table 2). The proportion of the $D_{2L}$ variant among the population of D2 transcripts examined in all the striata was about 2.3%, compared to 18% for $D_{2S}$ and 79% for $D_{2L}$ (Table 2), the latter two values generally agreeing with those found by others in human striata [31] and rat striata [12,26,45]. Although no cellular location of $D_{2L}$ was done in the present study, such work will be important to do, considering that $D_{2S}$ predominates in dopamine cell bodies axons while $D_{2L}$ is more strongly expressed by neurons in the striatum and nucleus accumbens [18].

A unique feature of the dopamine $D_{2L}$ receptor is that it arises from an aberrant splicing pattern of the RNA. Virtually all known introns have an AG sequence at their 3' ends (the acceptor site) [25], including all the introns spliced out in $D_{2L}$ [7,9,13]. However, intron 5 for $D_{2L}$ ends with a unique TG sequence, as illustrated in Fig. 2. Of the many thousands of known genes, no other intron has a 3' acceptor site which ends in TG. There are only two other proteins where the normal AG acceptor site has mutated into a TG. In these instances, however, the mutation results in either the exon being skipped (as in hypoxanthine phosphoribosyl-transferase in the Lesch–Nyhan syndrome) or the appearance of an abnormal splice site occurring within the exon (as in ornithine transcarbamylase deficiency), resulting in abnormal proteins being expressed, and resulting in disease in both cases [25]. Although the normal splicing reaction for introns ending in AG is done by the spliceosome with its 70 proteins and five small nuclear RNAs, the different ending of TG in the membrane, as extensively described elsewhere [see Ref. of Seeman and Van Tol, 1995, in Ref. [39]].

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other brain regions expressing dopamine receptors in health or in psychosis.

The low prevalence of this transcript variant is similar to the low prevalence of one transcript variant in 4000 normal transcripts for β-amyloid precursor protein and ubiquitin-B in Alzheimer’s disease [47,48].

The prevalence or expression of the transcript variants, moreover, may vary from time to time, depending on a variety of factors which affect gene expression. This would be consistent with the natural undulating course of psychotic symptoms in schizophrenia, where it has been observed that the symptomatology waxes and wanes for no apparent reason. Moreover, it is possible that the prevalence or expression of such transcript variants may be age-dependent [48], explaining why age is an important factor in the onset of psychosis.

There are several molecular mechanisms whereby transcript variants can result in abnormal neurotransmission. For example, the transcript variant can lead to the synthesis of an inactive receptor, as in the case of the dopamine D4Valine194Glycine variant [21,44]. Second, the transcript variant can produce an inherently active receptor in the absence of any activating ligand (i.e., a “constitutively active” receptor) [35]. Third, the mRNA transcript variants of a receptor can lead to the production of variant receptor proteins which can interfere with the function of the normal receptor. Dopamine D1, D2 and D3 receptors, for example, exist in the form of monomers or dimers [27,50].

Fig. 4. The dopamine D2noreg receptor variant has two extra amino acids, compared to D2norm, in the third cytoplasmic loop involved in coupling to intracellular proteins mediating the actions of dopamine. The amino moiety of dopamine (in red) attaches to aspartic acid (labeled D), while the two hydroxyls of dopamine attach to the two serines labeled S. Yellow: Phospholipid bilayer region of the cell membrane, with yellow spheres representing the head groups. Blue: Extracellular side of the membrane. Pink: Cytoplasmic side of the cell membrane. The amino acid chain of this D2 receptor protein starts on the extracellular side and traverses the cell membrane seven times, as indicated by the hydrophobic nature of the amino acids within the yellow–white hydrophobic region. The four cytoplasmic loops of amino acids serve to relay the receptors interactions with various intracellular messenger molecules such as G proteins and kinases. Amino acid code: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; A, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine (Copyright of main template, Seeman, 1990, 1991, 1996).
Thus, a dimer consisting of D2<sub>short</sub> and D2<sub>longer</sub> may not be fully active. Fourth, the transcript variant can produce receptor variants which can result in abnormal coupling to other proteins, such as G proteins or other proteins which bind to the SH domains of receptors. Such physiologic coupling generally occurs between the third cytoplasmic loop of the receptor and other proteins, especially the G proteins. In fact, the receptor variant reported here harbors a variation in the third cytoplasmic loop between the regions of transmembrane 5 and 6, as illustrated more clearly in Fig. 4.

In addition to the question of regional abundance of the transcript variant, the expression of the D2<sub>longer</sub> receptor protein may not quantitatively parallel the level of the transcript. For example, although the frontal cortex of the rat brain contains a relatively high level of dopamine D4 receptor mRNA, there is an absence of any D4-like specific binding in that region [36]. Moreover, although long-term antipsychotic medication generally elevates the density of dopamine D2 receptors by 30–50% in the rat striatum [38], the density of the dopamine D2 receptor mRNA usually exhibits little or no elevation [8,49]; in those cases where D2 mRNA has been found to be elevated, the elevation is short-lived [15]. Hence, despite the low prevalence of the dopamine D2<sub>longer</sub> receptor transcript variant found in the present study, the variant receptor protein itself may actually be higher in density than would be indicated by the low level of the variant mRNA.

Future research needs to examine the prevalence of such transcript and receptor variants in different brain regions (such as in Ref. [18]), the age-dependence of this prevalence, and, as well, the regulatory factors which may be responsible for creating transcript variants. In particular, because most of the known aberrant splicing patterns are associated with disease [1,25], future research must explore whether the splicing mechanisms in producing D2<sub>longer</sub> may sometimes be altered and associated with neuropsychiatric disease.

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