Schizophrenia: Normal Sequence in the Dopamine D_2 Receptor Region that Couples to G-Proteins. DNA Polymorphisms in D_2

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Because dopamine (DA) D_2 receptors are a target in neuroleptic therapy and have been found to be elevated in schizophrenia, the human DA D_2 receptor gene was examined for possible abnormalities in schizophrenia. Moreover, since D_2 receptors in psychosis have a reduced coupling to D_1 receptors, the cytoplasmic third loop of D_2 was chosen for deoxyribonucleic acid (DNA) sequencing, since this region is essential for coupling to G-proteins. This region also contains exon 5, which is expressed in the long form of D_2, but not in the short form of D_2. In eight schizophrenia cases, this region had normal exon sequences (exons 4, 5 and 6), and normal sequences at its intron-exon junctions. However, exon 6 contained three DNA polymorphic base changes, and introns 4 and 5 revealed three missing bases and two polymorphic base changes, none of which would be expected to alter the D_2 receptor protein in schizophrenia.

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KEY WORDS: *Schizophrenia; Dopamine D_2 receptor; Deoxyribonucleic acid sequence; Polymorphism; Polymerase chain reaction*

The human dopamine (DA) D_2 receptor is a candidate for examination of its possible genetic abnormalities in schizophrenia because the D_2 receptor is the common target of antipsychotic drug action (Seeman et al. 1976), with the single exception of clozapine, which targets to the DA D_4 receptor (Van Tol et al. 1991).

An additional reason for examining the D_2 receptor sequence is that D_2 receptors are consistently elevated in postmortem schizophrenia brain tissues (Seeman et al. 1987; reviewed by Seeman and Niznik 1990), suggesting that these receptors may have an abnormal molecular structure associated with their elevated density. The density of D_2 receptors is also elevated in postmortem schizophrenia tissues from never-medicated patients (Joyce et al. 1989). There is a bimodal pattern to these elevated D_2 densities (Seeman et al. 1984, 1987) insofar as half the postmortem tissues reveal D_2 densities beyond the normal range, and the other half reveal densities in the normal range.

Using [^{11}C]methylspiperone, positron-emission tomography (PET) also reveals elevated densities of D_2-like receptors in never-medicated living schizophrenic patients (Wong et al. 1986). Using [^{11}C]raclopride, Farde et al. (1990) found that D_2 receptors in the schizophrenic left putamen were significantly elevated by 2.8 pmol/mL compared to the schizophrenic right putamen. However, although the left schizophrenic putamen was also elevated by 3.2 pmol/mL when compared to control subjects, this elevation did not reach statistical significance. Using [^{11}C]raclopride to mea-

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sure D_2 receptors in never-medicated schizophrenic patients, Hietala et al. (1991) found that four of eleven patients revealed D_2 receptor densities elevated beyond the control group, although the overall densities between the two groups were similar. (The absolute densities of D_2 receptors reported by Hietala et al. were abnormally high, presumably a consequence of using the cerebellum as baseline, see below).

To help resolve these different findings obtained by PET, we examined the role of endogenous DA on the PET ligands. Both our in vitro and in vivo data clearly show that endogenous DA markedly lowers the apparent D_2 density when using [^{3}H]raclopride, but not when measured using [^{3}H]spiperone or [^{3}H]methylspiperone (Seeman et al. 1989a, 1990). Thus, radioactive raclopride underestimates D_2 in the presence of DA.

Furthermore, when the cerebellum is used as baseline (Farde et al. 1990), the apparent value for the total D_2 density is artificially raised, resulting in a further underestimate of the percent elevation in D_2 density (Seeman et al. 1990). These findings have been supported by Ross and Jackson (1989).

Although linkage between the D_2 receptor gene region and schizophrenia has been excluded in two schizophrenia pedigrees (Moises et al. 1991), schizophrenia is sufficiently heterogenous to warrant a search for possible D_2 receptor abnormalities in schizophrenic individuals from different pedigrees.

The present study, therefore, examined genomic deoxyribonucleic acid (DNA) from eight schizophrenic individuals. We focused on the midregion of the D_2 receptor (encoded by exons 4, 5, and 6), because this region is thought to associate with G-proteins (O'Dowd et al. 1988, 1989; Ohara et al. 1988), an association that appears disrupted or reduced in schizophrenia (Seeman et al. 1989). This region also contains an exon (exon 5), encoding 29 amino acids in the third cytoplasmic loop connecting the fifth and sixth transmembrane helices. This exon is expressed in the long form of D_2, but not in the short form of D_2.

We found the exon 4 to 6 region of the D_2 receptor to have a normal amino acid sequence in schizophrenia, but there were several polymorphisms.

MATERIALS AND METHODS

Tissues

Three schizophrenia striata were from the Lainz Hospital brain bank (Vienna), two schizophrenia striata and two control striata were from the Canadian Brain Tissue Bank (Toronto), and two blood samples were from consenting schizophrenic patients in the Toronto Schizophrenia Registry. The diagnostic criteria for schizophrenia were International Classification of Diseases, version 9 for tissues from Vienna, and DSM-III-R (American Psychiatric Association 1987) for cases from Toronto. Two tissue samples from two schizophrenics (T751 and T708) had previously been found to have a reduced link between D_1 and D_2 receptors (see companion paper by Ohara et al. 1993; Seeman et al. 1989).

DNA Amplification By the Polymerase Chain Reaction

Genomic DNA was extracted from each tissue sample (Ohara et al. 1992). As illustrated in Figure 1, the following regions of the D_2 receptor gene were selectively amplified in vitro by the polymerase chain reaction (PCR) using *Taq* polymerase with the oligonucleotide primers indicated:

1. Between the end of intron 3 and the start of intron 4 (using Primers 753 and 752), thus including exon 4, which contains the fifth transmembrane region of the D_2 receptor.

2. Between the end of intron 4 and the beginning of intron 5 (using Primers 61 and 60). This region includes exon 5, which encodes a 29-amino acid peptide, included in the long form of D_2, but excluded from the short form of D_2 (Grandy et al. 1989; Dal Toso et al. 1989).

3. Between the end of intron 5 and the beginning of intron 6 (using Primers 751 and 750), thus including exon 6, which contains a portion of the sixth transmembrane region of the D_2 receptor.

Attached to each of the six primers were an additional nine nucleotide bases (**tacggatcc**), encoding the *Bam*HI site (see Table 1). The PCR was done in tubes containing 200 ng genomic DNA, 1 μg of each primer, 200 μmol each of adenosine 5'-triphosphate, cytidine 5'-triphosphate, guanosine 5'-triphosphate, and thymidine 5'-triphosphate, and 2.5 U of *Taq* polymerase in a final total volume of 100 μl GeneAmp buffer (Perkin-Elmer Cetus Instruments, Norwalk, CT). Each PCR cycle consisted of 1.5 minutes at 95°C to denature the genomic DNA, 2 minutes at 60°C to permit the primers to anneal, and 3 minutes at 72°C for primer extension. A total of 30 cycles were done in a DNA Thermal Cycler (Perkin-Elmer Cetus Instruments). A final period of 7 minutes at 72°C was allowed for maximum DNA extension. An aliquot (60 μL) of PCR-amplified DNA was subcloned into SP73 (Promega) and subsequently sequenced by the standard dideoxy chain termination method (see Ohara et al. 1992 for references).

RESULTS

The PCR-amplified DNA from the control and schizophrenia tissues, when run on agarose gel, revealed identical sizes for each fragment amplified (data not shown). The Southern blots, probed with exon 5, did

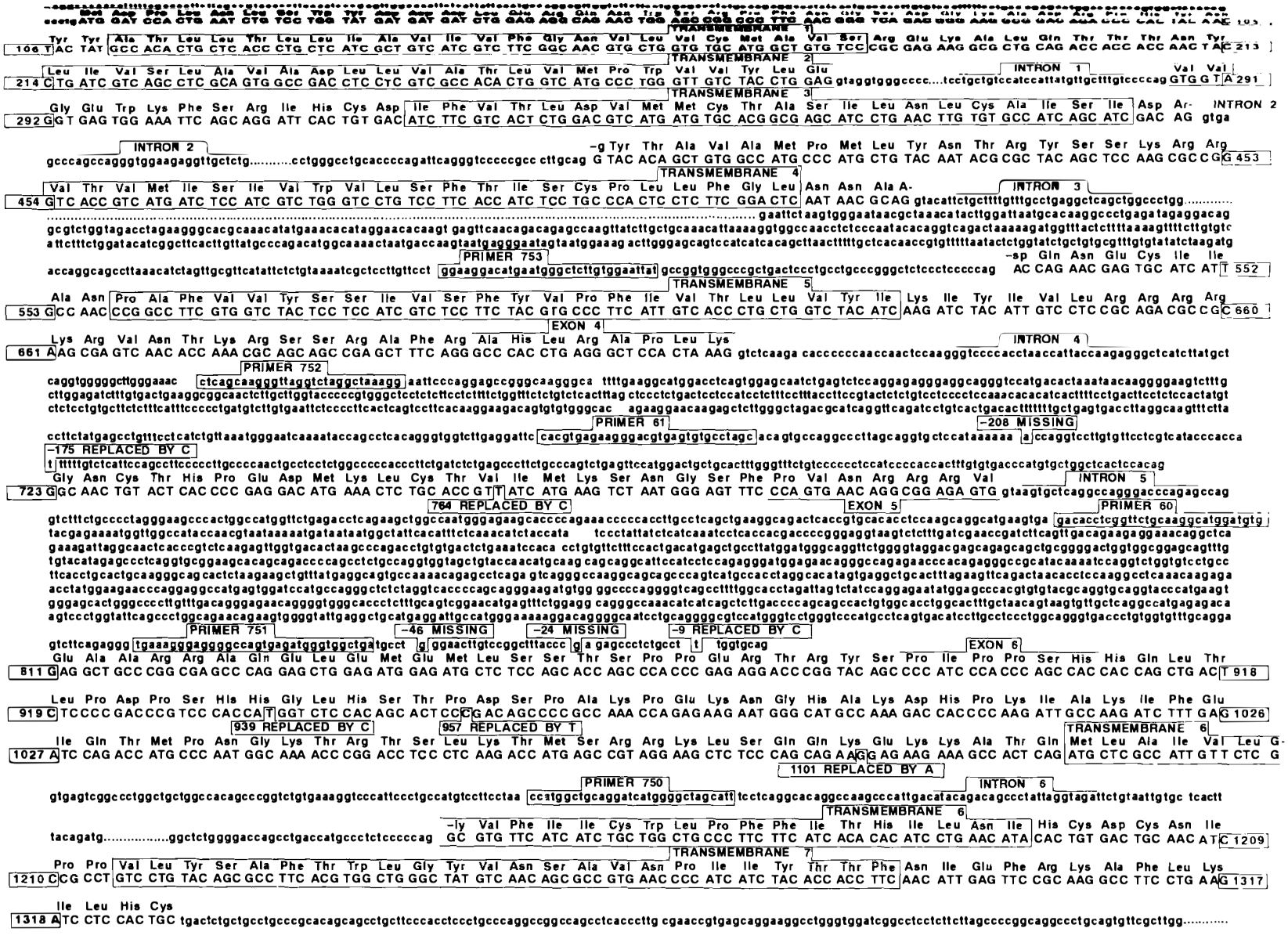

Figure 1. Map of the human DA D_2 receptor, amalgamated from Grandy et al. (1989) and Dal Toso et al. (1989). Lower case letters indicate noncoding bases; capitals indicate coding bases. Only the coding bases are numbered, using the scheme of Grandy et al. (1989). Primers 753, 752, 61, 60, 751, and 750, used to sequence the D_2 receptor in schizophrenia, are boxed. Missing or replaced bases are indicated, but see Table 1 for which individuals revealed changes. Boxed amino acids indicate the seven hydrophobic transmembrane regions of the D_2 receptor.

Table 1. Primer Sequence. The First Nine Nucleotide Bases (in Lower Case) Encode a *Bam*HI Site. Primers 752, 60, and 750 are Complementary to the DNA Coding Sequence of the Region Indicated in Figure 2

Primer Name	Sequence (5′ to 3′)	Region
753	tacggatccGGAAGGACATGAATGGGCTCTTGTGGAATTAT	Intron 3
752	tacggatccCCTTTAGCCTAGACCTAACCCTTGCTGAG	Intron 4
61	tacggatccCACGTGAGAAGGGACGTGAGTGTGCCTAGC	Intron 4
60	tacggatccCACATCCATGCCTTGCAGAACCGAGGTGTC	Intron 5
751	tacggatccTGAAAGGGAGGGGCCAGTGAGATGGGTGGCTGA	Intron 5
750	tacggatccAATGCTAGCCCCATGATCCTGCAGCCATGG	Intron 6

not reveal any differences between the control and schizophrenic tissues.

The PCR-amplified DNA was sequenced and compared to the previously published sequence of D_2 (Grandy et al. 1989; Dal Toso et al. 1989). The observed changes are shown in Fig. 1 and Table 2. None of these changes would result in a different amino acid sequence.

DISCUSSION

The polymorphic changes in the nucleotide sequences on both sides of exon 5 in both normal and schizophrenia individuals are unlikely to influence the splicing of exon 5. It should be noted the *Taq* polymerase has a base misincorporation rate of about 0.1%. It is possible, therefore, that an apparent DNA polymorphism may arise as a PCR-generated artifact. Other types of PCR procedures can be done to rule out such artifacts. It should also be noted that the numbering here of exons 4, 5, and 6 follows that of Grandy et al. (1989), but corresponds to exons 5, 6, and 7, respectively, of Mack et al. (1991, see references therein).

Exons 4, 5, and 6 of the D_2 receptor encode the so-called third cytoplasmic loop, a region that couples to G-proteins. Since these exon sequences were normal in the tissues examined here, it suggests that possible abnormalities in D_2 coupling to G-proteins in schizophrenia (Seeman et al. 1989) would not likely be based on the structure of this region of the D_2 receptor. However, additional research needs to be done to examine all the D_2 exons and all the intron-exon junctions.

The number of different samples tested here was small. Hence, the results do not preclude an abnormal amino acid sequence in the D_2 DA receptor of other, different patients. It may be calculated that studying eight subjects is not sufficient to rule out a receptor mu-

Table 2. Changes in the Nucleotide Sequence of the Human DA D_2 Receptor in Schizophrenia and Control Individuals, Compared to the Published Normal Sequence

	Intron 4			Intron 5					
	−208 Before	−175 Before	Exon 5	−46 Before	−24 Before	−9 Before	Exon 6		
Base:	Exon 5	Exon 5	764	Exon 6	Exon 6	Exon 6	939	957	1101
Grandy et al.	A	T	GTT	G	G	T	CAT	CCC	AAG
Amino acid:			Val				His	Pro	Lys
SZ V185-80	absent	C	T			C	C	C	G
SZ V232-81			T		absent	C	C	C	G
SZ V375-83	absent	C	T	absent	absent	C	C	T	G
									A
SZ V433-88	absent	C	T		absent	C	C	C	G
SZ T 708			T						
SZ T 751			T						
SZ TB 1	absent	C	T		absent	C	C	T	G
SZ TB 2	absent	C	C						
Control T545					absent	C	C	C	G
Control T590	absent	C	T						
Control T813		C	T	absent	absent	C	C	C	G
Dal Toso et al.							C	C	G
Selbie et al.							C	T	G
Stormann et al.							T	C	G
Sarker et al.					absent	C	C/T		

tation in possibly one-third of schizophrenic patients. A more rapid method for screening possible mutations in a larger series of tissues is to use PCR and denaturing gradient gel electrophoresis (Sung et al. 1991; Catalano et al. 1991).

The absence of changes in the D_2 receptor protein in these schizophrenia cases does not rule out other types of possible D_2 receptor abnormalities in schizophrenia (abnormal D_2 receptor phosphorylation and glycosylation, for example).

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