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Transcription of a human dopamine D5 pseudogene

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We have previously reported that the human genome contains the two pseudogenes ψDRD5-1, and ψDRD5-2, and that each share 94% homology when compared with the functional gene DRD5. There is only 2% difference at the nucleotide level between the two pseudogenes. We questioned whether these pseudogenes were transcribed, since transcription of either of these pseudogenes could result in false interpretation of in-situ hybridization and Northern blot analysis, using the DRD5 as a probe. We now report that we have detected transcription of one of the pseudogenes, ψDRD5-1, in several human brain areas, and this mRNA transcript is capable of producing a protein of 154 amino acids. Furthermore we report that PCR amplification of DRD5 or the pseudogenes in human tissue can result in the formation of chimer artifacts due to the co-amplification of three very similar genes.

The human genome contains five genes encoding human dopamine receptors, namely DRD1 (1, 2, 3, 4, and also recently reviewed in 5), DRD2 (6), DRD3 (7), DRD4 (8), and DRD5 (9) and two genes very similar to DRD5 encoding the pseudogenes ψDRD5-1, and ψDRD5-2 (10). Each of these pseudogenes contains five identical defects in the sequence, two stop codons and three frame shifts, that render these genes incapable of encoding functional receptors, shown in fig. 1A.. Only 2% difference at the nucleotide level between the pseudogenes suggests that the emergence of two ψDRD5 loci is a recent event in mammalian evolution. We have reported that the pseudogenes are not present in the genome of the African green monkey (Cercopithecus aethiops) and may only be present in human and closely related primates (10). We now report that one of these DRD5 pseudogenes is transcriptionally competent in several regions of human brain and we also report the presence of at least one DRD5 related pseudogene in the genome of gorilla.

Abbreviations; PCR, polymerase chain reaction; TM, transmembrane; DRD5, dopamine D5 receptor; DRD5, gene encoding the dopamine D5 receptor.

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Materials and Methods

DNA amplification by PCR and subcloning: The PCR methods were as described previously (10, and 11). In short, the human DNA sample was submitted to 30 cycles in the PCR with oligonucleotides A1 (5' TCCAGCCTGAATCGAACCTAC 3') and B1 (3' ACGTACCAGGGAAAGACGTCA 5'). The timing for each cycle was 1.5 min at 93^{0}C, 2 min at 55^{0}C , 4 min at 72^{0}C, followed by a 7 min extension at 72^{0}C. After 30 cycles this DNA was phosphorylated with T4 polynucleotide kinase and the ends were flushed using the klenow enzyme. This DNA was subcloned into the SmaI restriction endonuclease site of SP 73 (Promega) and transformed into E. coli (AG1 cells).

Isolation of mRNA, and cDNA preparation: Poly A$^+$ RNA was isolated from the SK-N-MC cell line essentially as described previously (12 and 13). Reverse transcription of poly A$^+$ RNA (1.0μg) into cDNA using either random primers or oligo dT with Moloney Murine Leukemia Virus RNase reverse transcriptase (Superscript, BRL) was performed as described by the supplier.

Genomic DNA extraction from gorilla hair roots: Ten hair roots from a male gorilla were digested in 0.5 ml extraction buffer (10mM Tris base, pH 8.0, 10 mM disodium ethylenediaminetetratacetate (EDTA), 10mM sodium chloride, 4mM dithiothreitol, 2% sodium dodecyl sulphate, and 1mg/ml of proteinase K) at 56^{0}C, overnight (14). Undigested hair shafts were removed and phenol (0.5 ml) added. The solution was mixed and centrifuged for 20 sec to separate the phases. The supernatant was removed, extracted with chloroform (0.5 ml), centrifuged for 2 min, and the supernatant collected. Absolute ethanol (1 ml) was added and the sample stored at 20^{0}C for 1 hr. The sample was centrifuged for 10 min, the pellet was dried under vacuum, and taken up in 20 μl of TE8 buffer (10mM Tris Cl, pH 8.0, and 1mM EDTA). The DNA sample was subjected to PCR amplification with oligonucleotides (5' AACATGACCAACGTCTTC 3') and (3' ACGTACCAGGGAAAGACGTCA 5'). The amplified DNA was subcloned as described above.

Results and Discussion

PCR amplification of a cDNA putamen library: The nucleotide sequence of ψDRD5-1 contains two BglII sites separated by 550 bp (10), whereas ψDRD5-2, and DRD5 contains only one of these BglII sites, illustrated in fig. 1B (10). We have used this restriction site difference to search for and locate

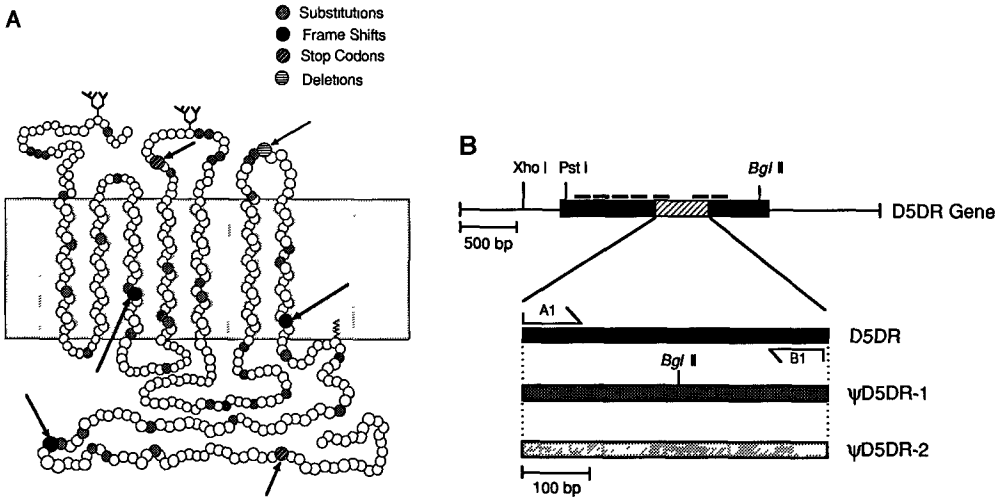

Figure 1. A. A representation of the topography of DRD5. The position of the two stop codons, the three frame shifts, and substitutions compared to DRD5 present in the equivalent position of ψDRD5-1, are indicated.
B. Strategy used in PCR amplification of the pseudogenes with the oligonucleotide primers, A1 and B1. The unique BglII site present only in the ψDRD5-1 PCR product is indicated.

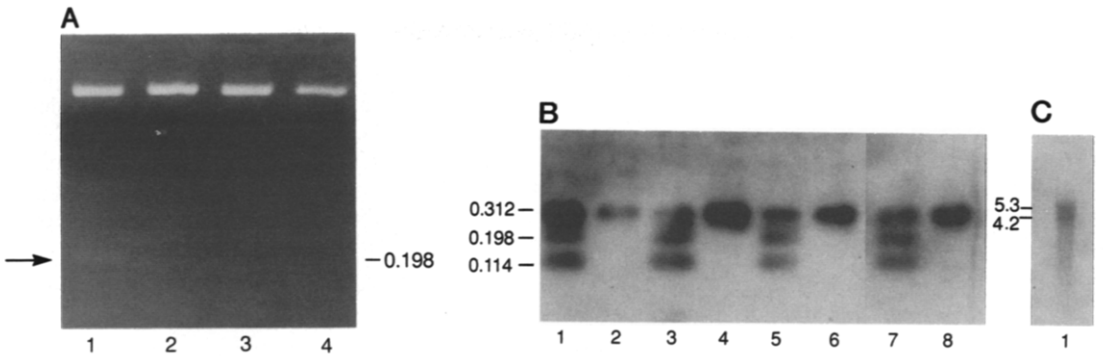

Figure 2. Analysis of the PCR products, amplified from human cDNA libraries, for the presence of a BglII restriction endonuclease site.
A. Minipreparations of the subclones obtained by PCR amplification from cDNA of putamen, using oligonucleotides A1 and B1. The amplified DNA was subcloned into SP73, digested with BglII and electrophoresed in agarose and stained with ethidium bromide.
B. PCR amplification using the two oligonucleotides, A1 and B1, followed by digestion with BglII cleaves the DNA fragment (size 312 bp) into the two smaller fragments (of size 198 and 114 bp). DNA samples cut with BglII: lane 1 (hippocampus), lane 3 (nucleus accumbens), lane 5 (substantia nigra), and lane 7 (putamen). DNA samples not cut : lane 2 (hippocampus), lane 4 (nucleus accumbens), lane 6 (substantia nigra), and lane 8 (putamen).
C. Northern blot analysis of mRNA (10µg of polyadenylated RNA) isolated from the human neuroblastoma cell line, SK-N-MC. The blot was hybridized with the ^{32}P-labelled 1.2 kb gene fragment gene fragment DRD5 as described (9) and exposed for three days at -70^{0}C to X-ray film with an intensifying screen.

cDNA clones encoding the pseudogene ψDRD5-1. Two oligonucleotides, A1 and B1, based on the sequence of ψDRD5-1 (10), flanking the unique BglII site, were used to prime a λgt11 cDNA putamen library (Clontech), fig. 1B. The amplified PCR products, (in the size range of 300bp) were subcloned into the plasmid SP73. Inserts were digested with BglII, resolved by agarose electrophoresis and stained with ethidium bromide. Two populations of plasmids were observed those that were linearised, fig. 2A, lane 2, 3, and 4 (sequence analysis of several of these plasmids demonstrated that they contained the PCR product of DRD5) and those that contained a 300 bp insert, fig. 2A, lane 1.

Formation of chimer PCR products: Sequence analysis of several of the plasmids with the BglII insert revealed a nucleotide sequence almost identical to the pseudogene ψDRD5-1. There were several small discrepancies in the nucleotide sequence of the clones compared to the previously reported sequence of ψDRD5-1 which was isolated from a human genomic library (10). We now realize that PCR amplification, in either the cDNA libraries or with human genomic DNA, with oligonucleotides homologous to the two very similar pseudogenes, and DRD5 can result in the formation of chimer PCR products, some of which are demonstrated in fig. 3. These PCR artifacts are created as a result of heteroduplex formation in the co-amplification of very similar genes, followed by transformation and repair in bacteria (this problem has been reported previously in other systems, Ref. 15). The variety of cDNA clones we obtained may have been formed as a result of co-amplification of all three genes in the putamen library ψDRD5-1, ψDRD5-2, and DRD5, or only DRD5 and ψDRD5-1, as yet we have not deteremined whether ψDRD5-2 is transcribed. To avoid the formation of chimer artifacts, PCR amplification of ψDRD5-1, ψDRD5-2, or DRD5 from either genomic DNA or cDNA

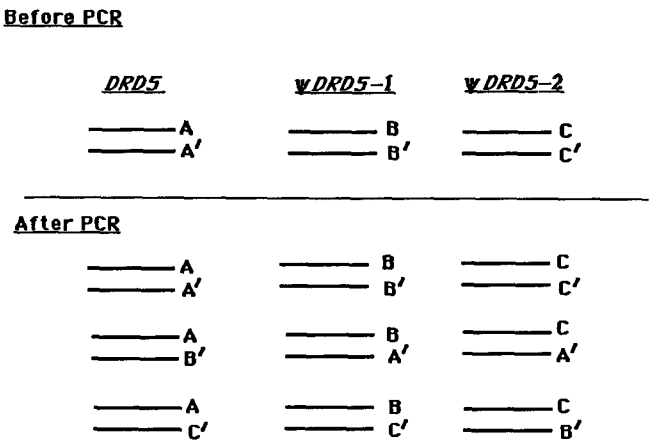

Figure 3. PCR amplification with oligonucleotides based on the three homologous genes, DRD5 and ψDRD5-1, ψDRD5-2, can result in the formation of multiple chimeric clones, some of which are illustrated.

will require the use of primers and regions more specific for each of these genes. The apparent lower number of plasmids in the putamen cDNA library encoding ψDRD5-1 compared to DRD5 (only 1 clone in 20), may be a result of either heteroduplex formation and removal of the BglII site in some percentage of the clones, mutations in the promoter region of ψDRD5-1 or reduced stability of the mRNA encoding ψDRD5-1.

Transcription of ψDRD5-1: We surveyed for the presence of ψDRD5-1 in human cDNA libraries (Clontech), including hippocampus, nucleus accumbens, and substantia nigra, as well as cDNA prepared from poly A^+ selected RNA isolated from the neuroblastoma cell line SK-N-MC (ATCC). Aliquots from each library, including putamen, and the cDNA from the cell line, were subjected to amplification in the PCR with oligonucleotides, A1 and B1 (fig. 1B). The PCR products obtained were divided in two, and one half was digested with BglII, electrophoresed, Southern transferred and probed with a DNA fragment encoding ψDRD5-1. The resulting patterns indicated that each of the cDNA libraries, fig. 2B, and the cDNA prepared from the neuroblastoma cell line contained clones encoding the pseudogene ψDRD5-1. These results indicate that mRNA encoding ψDRD5-1 is produced in these tissues.

A Northern blot prepared from mRNA isolated from the SK-N-MC cell line revealed two main bands (sized at 5.3 and 4.2 kb) when probed with the gene fragment encoding DRD5, fig. 2C. One of these mRNA bands is due to transcription of ψDRD5-1, and we are now investigating whether DRD5 and ψDRD5-2 are also transcribed in this cell line, also under investigation is why each of these mRNA transcripts from SK-N-MC cell line is larger than our estimate of the two DRD5 mRNA bands (sized at 3.3 and 3.5 kb) isolated from normal human brain tissue (9). The mRNA produced from ψDRD5-1 is probably translated forming a polypeptide of 154 amino acids, a receptor truncated as a result of a frame shift in the loop between TM3 and TM4 (fig.4). The fate and function of this polypeptide is unknown at present, but it is possible that it is unstable and removed rapidly by

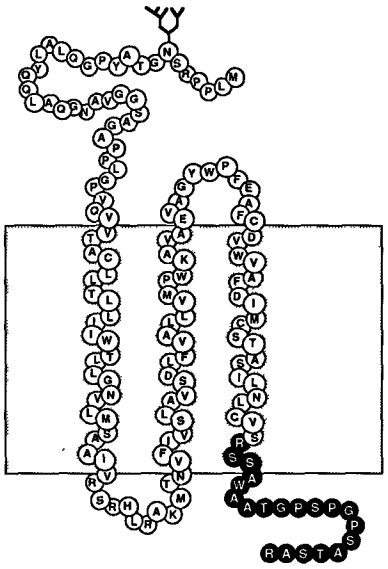

Figure 4. Representation of the truncated receptor encoded by ψDRD5-1. Amino acids (indicated in black), if translation proceeded through the first frame shift, due to an 8 bp insertion in the reading frame (10).

proteases, although truncated β_2-adrenergic receptors, with only 2 or 5 TM regions present, were found to be stable and inserted in the cell membrane (16).

PCR amplification of gorilla DNA: We have previously reported that we were unable to find pseudogenes in genomic DNA of the African green monkey (10) using oligonucleotides based on the sequence of human DRD5. PCR amplification of gorilla genomic DNA primed by two DRD5 specific oligonucleotides yielded two gene fragments, and each of these genes have strong homology with human DRD5, a partial sequence of the deduced is shown, (fig. 5). One of these clones shares the same deleterious mutation of a stop codon in the coding sequence as human ψDRD5 (fig. 5) thus present evidence indicates that DRD5 pseudogenes are present in the gorilla. In future experiments we wish to determine if pseudogene mRNA transcripts can play a role in regulating the transcription of DRD5.in human and related primates.

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1. His Arg Asp Gln Ala Ala Ser Trp Gly Gly Leu Asp Leu Pro Asn
2. His Arg Asp Gln Ala Gly Ser Trp Gly Gly Leu Asp Leu Thr Asn
3. His Arg Asp Gln Ala Ala Ser Trp Gly Gly Leu Asp Leu Pro Asn
4. His Arg Asp Gln Ala Val Ser --- Gly Gly Leu Asp Leu Pro Asn
5. His Arg Asp Gln Ala Val Ser --- Gly Gly Leu Asp Leu Pro Asn
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Figure 5. A partial sequence of the amino acids encoded by the genes amplified from gorilla genomic DNA compared with the human and monkey sequences. The sequences compared are as follows: 1. human DRD5, 2. monkey DRD5, 3. Gorilla DRD5, 4. human ψDRD5-, and gorilla ψDRD5. The region compared is located in the short loop between trasmembrane 4 and 5 in human DRD5 (9). The dashes in the sequence boxed indicate the position of the stop codon.

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