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IDENTIFICATION BY SEQUENCE ANALYSIS OF A SECOND RAT BRAIN cDNA ENCODING THE DOPAMINE (D2) RECEPTOR

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A rat brain cDNA library constructed in lambda ZAP II was screened with three oligonucleotide probes based on the reported coding region of the D2 receptor gene, RGB-2. A complete cDNA clone, D2(8)-1, showing positive signals with the three probes was subsequently identified by restriction analysis and dideoxy sequence analysis to be a variant of the RGB-2 gene. Comparison of the two genes revealed almost complete homology except that D2(8)-1 contains an 87 bp insert within the protein coding region and 265 additional nucleotides 5′ upstream from the 5′end reported for RGB-2. It is suggested that at least two mRNA species encoding for D2 receptors exist in rat brain, possibly resulting from alternative splicing of RNA.

The actions of dopamine (DA) in the nervous system are mediated by at least two types of receptors. DA receptors that mediate a positive cyclic AMP response are termed D1, while DA receptors that are negatively coupled to adenylate cyclase (AC) are termed D2 (1). Most neuroleptic drugs bind preferentially to D2 receptors (2). It has been definitively established that chronic neuroleptic treatment in animals produces an increase in the number of D2 receptors in brain (2) and that DA receptor agonist treatment decreases the number of D2 receptors (3). The ability of the D2 system to produce homeostatic increases in receptor number may have physiological importance other than in response to D2 blockade by drugs (4). Thus, studies directed toward understanding the regulatory properties of these receptors have broad implications. Our progress toward this goal has been significantly advanced by the isolation and characterization of a brain cDNA (RGB-2) encoding for the D2 receptor (5, 6). In this report, we describe the cloning and sequencing of a variant D2 receptor cDNA from rat brain.

MATERIALS AND METHODS

Three oligonucleotides corresponding to three areas of the coding region of RGB-2 (5)) were synthesized with Applied Biosystems instrumentation and purified by Sephadex G-50 chromatography: P1 (5′-CTGCCTCTCCAGATCGTCATCGTACC-AGGACAGGTTCAGTGGAT-3′); P2, (5′-CCGGGTCCTCTCTGGGGGGCTGGTGCTTGACAGCATCTCC-

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ATTT-3'); P3 (5'-GCAGTGCAAGATCTTCATGAAGGCCTTGCGGAACTCGATGTTGAA-3'). A rat brain (Sprague-Dawley), lambda ZAP II cDNA library (2 x 10^6 recombinants), prepared using an oligo dT primer was carried through one amplification round. The amplified library was screened with the three probes using standard plaque hybridization procedures (7). The filters were washed for 1.5 hrs in 3 x SSC (1 x, 0.15 M NaCl and 0.015 M citrate) and 0.1% sodium dodecyl sulfate (SDS) at 65°C, then prehybridized for 2 hours in 3 x SSC, 5 x Denhardt's, 0.05% sodium pyrophosphate, 100 ug/ml denatured salmon sperm DNA and 0.5% SDS at 37°C. Hybridization was carried out with P1, P2 or P3, (3'-end labeled with ^{32}P-dideoxy ATP) in 3 x SSC, 1 x Denhardt's, 0.05% sodium pyrophosphate and 150 ug/ml denatured salmon sperm DNA for 18-19 hours at 55°C. The specific activity of the probes was >7 x 10^7 dpm/ug. Filters were then washed to a stringency of 1 x SSC/0.1% SDS at 50°C. Selected clones were treated with R408 helper phage to excise the phagemid, pBluescript SK(-) containing the cDNA inserts from lambda ZAP II. Sequencing inserts were prepared by subcloning of restricted fragments, purified twice on agarose gels, in pBluescript SK(-) or pSP73 (Promega). Complete sequencing was accomplished using 20-mer oligonucleotide primers based on RGB-2 that were synthesized as above. Supercoiled plasmid DNA was alkali denatured, neutralized, precipitated followed by primer annealing and sequencing by the dideoxy method (8), using Klenow fragment and AMV reverse transcriptase.

RESULTS AND DISCUSSION

One clone, designated D2(8)-1, positive with all three probes was found to contain a 2.8 kb insert. Restriction analysis of D2(8)-1 was made using restriction endonucleases known to act on RGB-2. An apparent identity in the restriction map was found; however, sequencing of D2(8)-1 revealed the presence of an 87 bp segment within the protein coding region that was not found in RGB-2 (Fig. 1, A). The deduced amino acid sequence from this 87 bp insert is also seen in A. The presence of this 87 bp was confirmed by analyzing overlapping sequences using two 24-mer oligonucleotide probes designed as primers for this region. The longest open reading frame in D2(8)-1 encodes a 444 amino acid protein with a molecular weight of 50,909 daltons. An additional 265 nucleotides, upstream from the 5' end of RGB-2 were also found (Fig. 1, B) and the poly A+ tail at the 3' end was preceded by 11 additional nucleotides (Fig. 1, C). The restriction map for D2(8)-1 is also shown (Fig. 1, D).

The finding of this D2 cDNA variant strongly suggests the presence of two D2 receptor mRNAs and proteins, possibly resulting from alternative splicing of RNA. Recently, Snyder et al. (9) presented preliminary evidence for the existence of two mRNA species encoding the D2 receptor in rat. Using oligonucleotide primers designed for amplification of the coding region of the D2 gene in the polymerase chain reaction, they cloned two cDNA species, one about 1250 nucleotides and the other about 100 nucleotides longer. The cloning of human pituitary D2 receptors coded for by alternately spliced mRNAs has also been reported in a recent abstract (10). The functional differences between these D2 receptor variants remains to be elucidated.

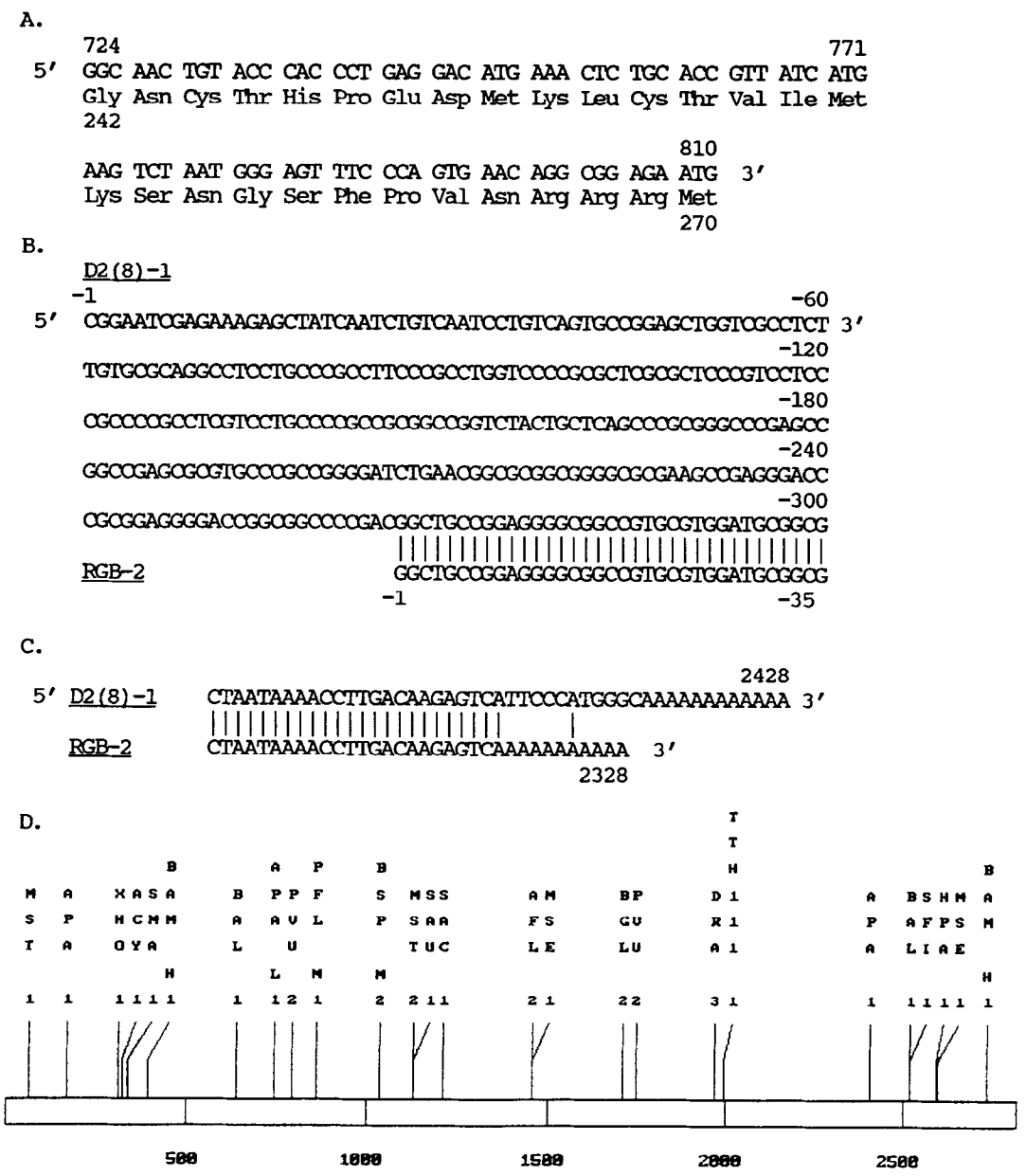

Fig. 1: A. The nucleotide and deduced amino acid sequence for the 87 bp insert found in the rat cDNA clone D2(8)-1. The 29 amino acid sequence is inserted at position 242 of the protein and position 724-810 of the nucleotide sequence; position 1 representing the first nucleotide of the protein coding area. B. The 5′-end of D2(8)-1 (-1 to -392) showing 265 nucleotides upstream from the 5′-end reported for RGB-2 (-266 to -392). C. The 3′-end of D2(8)-1 showing differences at the poly A+ tail with RGB-2. D. Restriction map of D2(8)-1, some enzyme sites not shown.

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