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Organization and Expression of the Rat $D2_A$ Receptor Gene: Identification of Alternative Transcripts and a Variant Donor Splice Site†

Karen L. O'Malley,*,‡ Kenneth J. Mack,‡ Kuan-Yun Gandelman,‡ and Richard D. Todd§
Department of Anatomy and Neurobiology and Departments of Psychiatry and Genetics, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110

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ABSTRACT: We have recently reported the creation of a cell line expressing D2 receptors encoded by a gene distinct from that described by Bunzow et al. [Bunzow, J. R., Van Tol, H. H. M., Grandy, D. K., Albert, P., Salon, J., Christie, M., Machida, C. A., Neve, K., & Civelli, O. (1988) *Nature 336*, 783–787]. To provide a framework for understanding structural differences between these and other G-protein-coupled receptors, the structure of the rat gene coding for the Bunzow et al. cDNA (called $D2_A$ here) was delineated. The $D2_A$ gene contains eight exons and spans at least 50 kb. Sets of oligonucleotide primers were used in combination with the polymerase chain reaction (PCR) to determine the presence of alternative transcripts within the introns. In contrast to other G-protein-coupled receptors, the $D2_A$ gene undergoes alternative RNA processing within intron 5, resulting in an insertion of 29 amino acids to the predicted 415 amino acid sequence of the $D2_A$ protein. By use of the PCR assay the relative abundance and tissue distribution of the alternative $D2_A$ transcripts (herein termed $D2_{A415}$ and $D2_{A444}$) were determined. A variant donor splice site was also identified at the end of exon 4, a GC dinucleotide instead of the canonical GT. The variant dinucleotide was also present in the mouse but not in the human $D2_A$ gene.

Dopamine receptors have been widely studied due to their proposed roles in the treatment and etiology of many neuropsychiatric disorders. Pharmacological and physiological studies have defined two principle types of dopamine receptors, D1 and D2, each with distinct pharmacological binding profiles, signal transduction systems, and sites of localization (Hamblin et al., 1984; Seeman et al., 1985; Kebabian, 1986; Stoof & Kebabian, 1984; Freedman & Weight, 1988; Enjalbert et al., 1988). Bunzow et al. (1988) have reported the cloning of a rat cDNA with the expression characteristics of a D2 receptor. This clone is a member of the G-protein[1]-coupled receptor family.

Recently we described a strategy for cloning cell surface proteins for which only radioligands are available (Todd et al., 1989). Using this technique, we isolated a cell line expressing a membrane-bound protein with the pharmacological characteristics of a D2 receptor. With polymerase chain reaction (PCR) analysis we have shown that the expressed D2 receptor is not the product of the Bunzow et al. D2 receptor gene (Todd et al., 1989). Therefore, there must be at least two genes that produce D2 receptor subtypes designated here as $D2_A$ (Bunzow et al., 1988) and $D2_B$ (Todd et al., 1989).

In order to characterize the functional and evolutionary relationships between these receptors and as the first step in developing experimental systems for studying the regulation of D2 receptor gene expression during development and differentiation, we have isolated and characterized the rat $D2_A$ gene.

Experimental Procedures

Materials. Most enzymes were purchased from Promega Biotec. Sequanase and AmpliTaq were from U.S. Biochemicals. Nylon membranes were from Schleicher & Schuell. Radionucleotides were purchased from Amersham. A λ Dash Fisher rat genomic library was obtained from Stratagene Cloning Systems.

Isolation of a Rat $D2_A$ Gene. A 15-kb phage recombinant clone encoding exons 2–8 was isolated as described (Todd et al., 1989). Fragments from this clone were used to screen a rat genomic library. Subsequent 5′ walking clones were obtained with either a T_3 or a T_7 promoter to generate end-specific RNA probes as per the manufacturer's protocols. The exon 1 containing recombinant phage was isolated by hybridization with a 96-bp fragment encoding nucleotides 1–96 reported by Bunzow et al. (1988). Labeling of probes, hybridization, and washing conditions were performed in accordance with standard methods (Feinberg & Vogelstein, 1983; Maniatis et al., 1982).

Oligonucleotides. Oligonucleotide primers were synthesized by the Protein Chemistry Facility, Washington University, on an Applied Biosystems DNA synthesizer. Oligonucleotides used for this study were derived from the rat $D2_A$ receptor cDNA (Bunzow et al., 1988) and included the following:

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* Address correspondence to this author.
‡ Department of Anatomy and Neurobiology.
§ Departments of Psychiatry and Genetics.

[1] Abbreviations: bp, base pair(s); kb, kilobase; G-protein, guanine nucleotide binding protein: PCR, polymerase chain reaction.

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orD2_A-225, 5′(dGGCTGCCGGAGGGGCGGC), identical with nucleotides −127 to −110; orD2_A-215, 5′-(dCTTCAAGCCATATGGCGC), complementary to nucleotides −31 to −49; orD2_A-227, 5′(dTCCAGGTAGACAACCCACGGCATT), complementary to coding sequence 261–284; orD2_A-197, 5′-d(AACCTGTCCTGGTACGATGACGAT), identical with nucleotides 13–36; orD2_A-198, 5′-d(GCACATCATGACATCCAGAGTGAC), complementary to 331–354; orD2_A-236, 5′-d(TTGTTGAGTCCGAAGAGC), complementary to 496–513; orD2_A-211, 5′-d(TACACAGCTGTGGCAATG), identical with 397–414; orD2_A-222, 5′-(CGGAGGACGATGTAGATT), complementary to 633–650; orD2_A-232, 5′-d(TACATCGTCCTCCGGAAGCGCCGGAA), identical with 637–662; orD2_A-224, 5′-d(TGGTGGGATGGATCAGGGAGAGTGAG), complementary to 832–851; orD2_A-223, 5′-d(CAGGCAGAGGCCAGACTTGC), complementary to sequence 3′ of GC dinucleotide shown in Figure 2.

DNA Sequence Analysis. Oligonucleotides were synthesized from both strands of cDNA (Bunzow et al., 1988) at ~250-bp intervals. These probes were initially used to identify relevant genomic restriction fragments. After subcloning, exons and intron boundaries were sequenced on both strands with denatured double-stranded DNA as a template for primer-extended synthesis (Chen & Seeburg, 1985). DNA analysis was facilitated by computer programs created by Bionet.

Polymerase Chain Reaction. To obtain exon 1, 1 μg of rat genomic DNA was used as a template in amplification reactions following Perkin-Elmer/Cetus protocols. One hundred picomoles each of orD2_A-225 and -215 was used to prime synthesis. Reaction products were separated on a 12% polyacrylamide gel, and a fragment of the appropriate size was excised, electroeluted, and ethanol precipitated.

For amplification of RNA, 1 μg of total RNA (Chomczynski & Sacchi, 1987) was reverse transcribed (Krug & Berger, 1987) with the indicated primers with second-strand synthesis and further amplification under the conditions described above. Amplification temperatures for primer sets 225/215, 225/227, 197/236, 211/222, and 211/223 were denaturation at 93 °C for 2 min, annealing at 50 °C for 1 min, and synthesis at 72 °C for 2 min. Temperatures for primer sets 197/198 and 232/224 were 93 °C (1.5 min), 61 °C (1.5 min), and 72 °C (1.5 min). We determined 28 cycles was proportional to the starting material by removing aliquots of reverse-transcribed basal ganglion RNA primed syntheses at intervals of 15, 20, 25, 30, and 40 cycles. The bands visualized at 30 cycles were still within the exponential part of the amplification curve.

In order to increase the sensitivity of some PCR experiments, primers were end labeled and included in the reaction mixture at a specific activity of 5×10^4 cpm/pmol. Typically, products were electrophoresed on 5% polyacrylamide gels followed by autoradiography. The specificity of amplification was confirmed by transferring unlabeled PCR products to nylon membranes after electrophoresis (Nguyen, 1989). Blots were probed with oligonucleotides internal to the amplification primers. Autoradiographs were quantitated with a Biorad densitometer.

Results and Discussion

Organization of Rat D2_A Gene. A partial genomic clone encoding the rat D2_A gene was isolated as described (Todd et al., 1989). Overlapping recombinant phage clones were subsequently identified with end fragments and oligonucleotide probes derived from the rat D2_A cDNA sequence (Bunzow et al., 1988). Using conventional chromosome walking techniques, we isolated three additional overlapping phage clones without finding exon 1. This exon was subsequently isolated by screening with a 96-bp DNA fragment amplified from rat DNA by use of opposing primers. No overlap could be detected between this clone and the most 5′ walking clone from exon 2, indicating intron 1 is at least 25 kb in length. All exons and splice junctions were sequenced and aligned with the rat D2_A cDNA sequence (Bunzow et al., 1988). As shown in Figure 1, the rat D2_A gene spans at least 50 kb with exons 2–7 clustered in approximately 13 kb of genome.

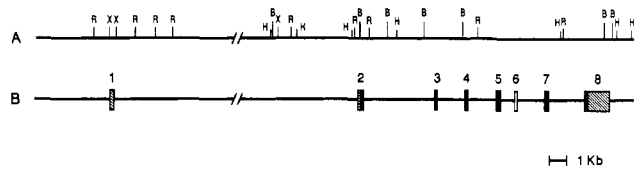

FIGURE 1: Organization of the D2_A gene. (A) Restriction map of the D2_A locus. The map is continuous except for a large gap (//) which remains in intron 1. Restriction sites: B, *Bam*HI; R, *Eco*RI; H, *Hin*dIII; X, *Xho*I. (B) Expressed exons are depicted by solid boxes, nonexpressed exons are depicted by hatched boxes, and the alternatively expressed exon is depicted by a clear box. Introns are represented by thin lines. Each intron/exon boundary was sequenced several times.

We sequenced 500 nucleotides preceding the start of the D2_A cDNA (Bunzow et al., 1988) but were unable to find transcriptional elements such as CCAAT or TATA boxes in characteristic positions. The region was ~78% GC and contained several SP1-like elements (Jones et al., 1985). We have determined that the D2_A mRNA is 2.9 kb (data not shown), which is in agreement with values extrapolated from Northern blots presented by Bunzow et al. (1988). Therefore, these sequences probably represent additional untranslated nucleotides that were deleted in the cDNA cloning process of Bunzow et al. However, we cannot rule out the possibility of an additional intron prior to the start of transcription. A third possibility is that the D2_A gene has an atypical promoter region, one which has characteristics of many housekeeping genes. Precise characterization of the transcription start site is in progress. Having sequenced all exons by use of both strands, we confirmed the cDNA sequence of Bunzow et al. (1988) except for several differences in the 3′ untranslated region. These include a G instead of an A at nucleotide position 1344, an A instead of a G at 1643, an A instead of a G at 1665, and a C instead of a T at 2297.

Variant Donor Splice Site. With one exception all intron/exon junctions contain canonical splice sequences (Table I; Mount, 1982; Ohshima & Gotoh, 1987). Sequencing of exon 4 revealed a GC dinucleotide beyond which identity with the cDNA diverged (Figure 2). A recent compilation of 5′ splice site sequences of 3294 introns indicates there are only 19 examples (0.58%) in which the second position of the GT dinucleotide has been substituted with either C or A (Jacob & Gallinaro, 1989). Two such examples, the chicken and duck α^D-globin gene (Fischer et al., 1984) and the murine αA-crystallin gene (King & Piatigorsky, 1983), have been shown to allow normal splicing.

To rule out sequencing artifacts, we synthesized an oligonucleotide complementary to the intronic sequence 3′ to the putative splice junction. Sequence primed with this oligonucleotide confirmed the GC observation (Figure 2). Additionally the acceptor site determined for exon 5 is at the predicted position to generate an in-frame transcript only if the GC splice is utilized (Table I). To rule out genomic cloning artifacts and to determine the specificity of the novel donor junction, we isolated mouse and human D2_A receptor genes (Todd et al., 1989; Gandelman, Mack, Todd and O'Malley,

Table I: Splice Sites of D_{2A} Gene[a]

Exon No.	Size (bp)	Exon/Intron Junctions: Donor		Acceptor	Intron Size, kb
1	>96	TTGAAG^{-30}	gtaagaaccggc	tctcctggccag $^{-31}$AGCCGT	>25
2	316	CTGGAG^{285}	gtggtgggtgag	ttttatctccag ^{286}GAGGTG	~3.2
3	111	TGACAG^{395}	gtgaggacaaca	ttgcctttgcag ^{396}GTACAC	~1.5
4	137	ATACAG^{532}	*gcaagtctggcc	ctattcccccag ^{533}ACCAGA	~1.7
5	191	CTCAAG^{723}	gtctctgacctc	ctcacttcacag $_{724}$GGCAAC	~0.9
6	87	AGAATG$_{811}$	gtaagtgttcag	tgtctgccacag $^{724}_{812}$GATGCT	~1.4
7	331	TTCTCG$^{1054}_{1141}$	gtgagtcagctc	cccttttctcag $^{1055}_{1142}$GTGTGT	~2.3
8	1263	CACTGC	...aataaaccttgacaagagtcattccatggtcacttgggtggctggaacactgt		

[a] Exon sequences are in upper-case letters; 3′ untranslated and intron sequences are in the lower-case letters. Superscript numbers represent nucleotide positions of exon/intron boundaries in the $D2_A$ cDNA sequence (Bunzow et al., 1988). The alternative transcript, $D2_{A444}$, encoded by exon 6 is described in the text. Numbering of subsequent nucleotide positions including this insert is in subscript. Consensus polyadenylation signals are underlined. An arrowhead indicates the polyadenylation site; an asterick indicates the variant donor splice site.

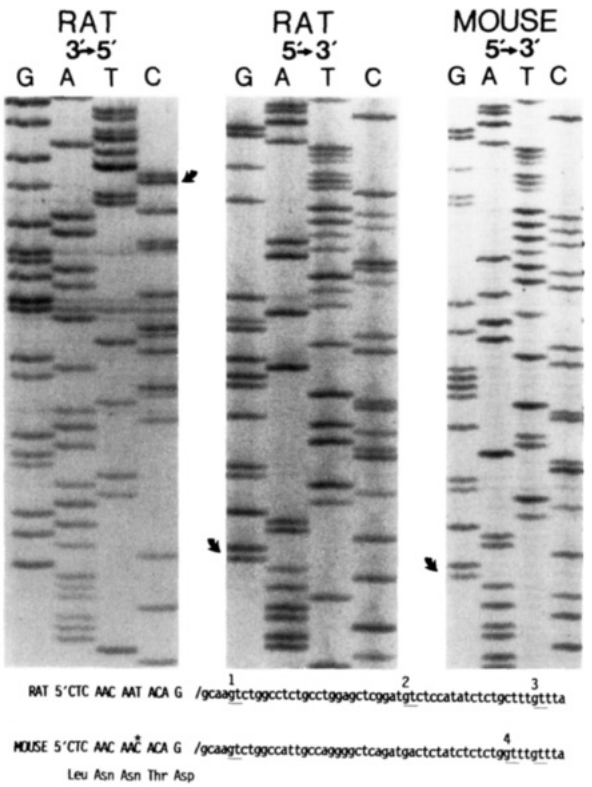

FIGURE 2: Sequence of variant donor dinucleotide from rat and mouse. Exon 4 containing subclones from rat and mouse $D2_A$ genes here sequenced 5′–3′ with $orD2_A$-211 as a primer. The 3′–5′ rat sequence was obtained with an oligonucleotide complementary to the intron sequence 100 bp downstream of the exon 4 splice site. The variant GC dinucleotide is indicated by an arrow; the single nucleotide difference between rat and mouse coding sequence is marked with an asterick. The derived sequence is shown below with exon sequence in upper-case letters and intron sequence in lower-case letters. Downstream GT dinucleotides are numerically labeled.

unpublished observation) and sequenced this region. As in the rat, the mouse $D2_A$ receptor gene also contains a GC dinucleotide at the exon 4 boundary (Figure 2). The human gene, however, contains the usual GT donor dinucleotide at this position.

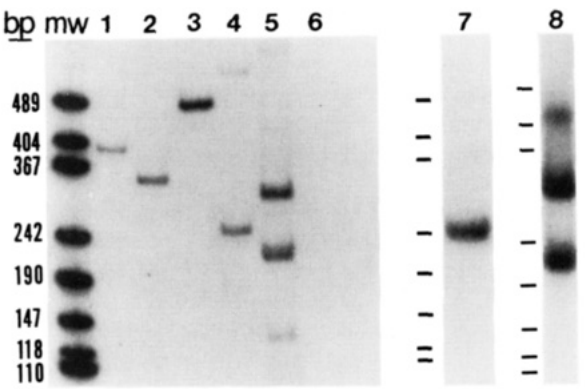

FIGURE 3: PCR analysis of rat $D2_A$ spliced junctions. Origin of rat $D2_A$ alternative transcripts was determined by reverse transcribing basal ganglion RNA with exon 2–7 complementary primers. The resulting cDNAs were subsequently amplified with the appropriate end-labeled primer sets shown in Table II. PCR products were separated on a 5% polyacrylamide gel, dried down, and autoradiographed. Lane 1, PCR reaction product generated by primer set $orD2_A$-225/227 flanking intron 1; lane 2, reaction products derived from $orD2_A$-197/198 flanking intron 2; lane 3, band generated with $orD2_A$-197/236 crossing both introns 2 and 3; lane 4, $orD2_A$-211/222; lane 5, $orD2_A$-232/224; lane 6, $orD2_A$-211/223. Lane 7 is identical with lane 4 except that the amplification primers $orD2_A$-211/222 were unlabeled. Following electrophoresis this lane was cut out, transferred to nylon, and then hybridized with $orD2_A$-236, a primer internal to those used for amplification. Lane 8 is identical with lane 5 except that the amplification primers $orD2_A$-232/224 were unlabeled and the separated products were hybridized with $orD2_A$-218, an internal exon 7 probe.

Since additional "GT" sequences are present 3′ of this site (Figure 2), the possibility of cDNA cloning artifacts was raised. To test this hypothesis, we used PCR amplification in combination with reverse-transcribed basal ganglion RNA. An oligonucleotide complementary to sequences in exon 5 ($orD2_A$-222) was used to prime cDNA synthesis and subsequent DNA amplification in conjunction with an exon 4 primer ($orD2_A$-211). If the variant donor splice site is utilized, a 253-bp fragment will be amplified from these primers (Table II). As shown in Figure 3 (lane 4), a band of 253 bp was observed, suggesting splicing had occurred at the GC dinucleotide to yield the predicted fragment. However, as shown

Table II: Predicted Size of PCR Fragments Using Indicated Primers[a]

primer sets	exon junction	size (bp)
orD2$_A$-225/227	1/2	411
orD2$_A$-197/198	2/3	344
orD2$_A$-197/236	2/4	500
orD2$_A$-211/222	4/5	253
orD2$_A$-232/224	5/7	214
orD2$_A$-211/223	4/intron 4[b]	155

[a] Sequences of primer sets are as described under Experimental Procedures. [b] The primer, orD2$_A$-223, is derived from intron 4 (Figure 2).

in Figure 2, there are several GT dinucleotides 3′ to the variant splice site. We can rule out their use because of the following: First, GT dinucleotides 1 and 3 would lead to "frame shifts" in the following coding sequence. Second, use of GT dinucleotide 2, although in frame, would create a PCR fragment of 283 base pairs, which was not observed (Figure 3, lane 4). Third, this GT dinucleotide is not present in the mouse, and GT dinucleotide 4 (Figure 2) is not present in the rat despite the 87% sequence identity in this region. In place of GT 2, the mouse sequence has a GA creating a stop codon in this position. Additional stop codons are found downstream of GT dinucleotide 3 in both rat and mouse sequences. Finally, an oligonucleotide complementary to the first 20 nucleotides of intron 4 (orD2$_A$-223) failed to prime cDNA synthesis and subsequent amplification of a predicted band, which would be expected if these sequences were present in the RNA pool (Figure 3, lane 6). Therefore, we conclude the variant GC dinucleotide is utilized to generate the observed mRNA species in rat and mouse.

Multiple Rat D2$_A$ Transcripts Are Derived from Intron 5. Transcripts from a number of different genes undergo alternative splicing of specific introns to form mRNAs that code for proteins with different properties. Recent reports suggest tht unlike other G-coupled protein receptors the D2$_A$ gene appears to undergo alternative RNA processing. Several investigators (Grandy et al., 1989; Snyder et al., 1989; Chio et al., 1989) have identified a D2$_A$ mRNA containing 87 additional nucleotides presumably arising via alternative splicing. The physiological significance and regulatory properties of this D2$_A$ isoform are unknown.

Because of the potential difference in regulatory properties and the possibility of other forms being present, we systematically examined each intron for the presence of alternative transcripts. Therefore, primer sets complementary to exons flanking introns 1–5 were used to coamplify alternative transcripts if present. Previously, we determined there are no alternative transcripts amplified from intron 7 (Todd et al., 1989). Table II lists the expected size of the PCR products. As shown in Figure 3, PCR products using intron 1–4 flanking primers all generated only a single primary band of the correct predicted size. In contrast primers flanking intron 5 coamplified two major bands of ~214 and 300 bp. On the basis of the predicted number of amino acids, we have called these D2$_{A415}$ and D2$_{A444}$.

We confirmed that these bands represented D2$_A$ mRNA, first, by restriction digests with exon-specific enzymes (data not shown) and, second, by transferring unlabeled PCR products to nylon membranes following electrophoresis. Blots were subsequently probed with an oligonucleotide internal to the amplification primers. The results for exon 4/5 and 5/6 PCR products are shown in Figure 3, lanes 7 and 8, respectively.

On longer exposures faint higher molecular weight bands were observed in lanes 2–4 (Figure 3). We determined these were artifactual since (1) they did not cut with the exon-appropriate enzymes (data not shown) and (2), when probed with internal primers, there is no hybridization (for example, lane 7 of Figure 3). In contrast to these results, the primary bands coamplified within intron 5 both hybridize with an internal primer (Figure 3, lane 8). Additionally, each of these bands is cut with *Sac*I, a unique restriction site at nucleotide 741 within exon 5 (data not shown). On the basis of single and double restriction digests, the faintly hybridizing band of 420 bp (Figure 3, lane 8) appears to be a PCR artifact.

The sequence D2$_{A444}$ was contained in a single additional exon (exon 6). Sequence analysis of exon 6 is in agreement with that reported for the cDNA by Chio et al. (1989), specifically 5′-GGCAACTGTACCCACCCTGAGGACATGAAACTCTGCACCGTTATCATGAAGTCTAATGGGAGTTTCCCAGTGAACAGGCGGAGAATG. Interestingly, the exon 6 donor splice site (Table I) is a perfect match to the consensus donor sequence AG/GTAAGT (Jacob & Gallinaro, 1989). In contrast, the donor splice site following exon 5 contains three mismatches. This poor match may contribute to the observed alternative splicing described below.

Tissue Distribution of Multiple D2$_A$ Transcripts. In order to address the hypothesis of differential expression of D2$_A$ mRNAs, we examined various central nervous system tissues

Table III: Normalized Proportions (%) of D2$_A$ mRNA by Brain Region[a]

	PCR product (size)				
tissue	D2$_A$ (344 bp)	D2$_{A444}$ (300 bp)	D2$_{A415}$ (214 bp)	D2$_{A444+415}$ (total)	D2$_{A444:415}$ ratio
medulla	53.4 ± 24.5	43.6 ± 14.9	118.4 ± 62.8	73.7 ± 33.7	0.5
pons	24.5 ± 8.3	10.4 ± 5.5	48.4 ± 27.5	25.7 ± 12.7	0.3
cerebellum	4.2 ± 4.3	4.5 ± 3.9	24.1 ± 10.4	12.4 ± 5.3	0.3
midbrain	34.4 ± 7.9	30.0 ± 6.0	70.2 ± 21.1	42.6 ± 11.3	0.5
hypothalamus	44.5 ± 22.4	25.6 ± 11.4	62.5 ± 17.9	40.5 ± 12.8	0.6
thalamus	37.9 ± 29.5	30.0 ± 12.2	62.3 ± 33.0	43.3 ± 20.1	0.7
anterior pituitary	170.0 ± 12.6	191.0 ± 8.0	37.2 ± 7.4	128.2 ± 6.2	7.6
amygdala	11.0 ± 12.2	19.8 ± 9.4	49.4 ± 29.0	31.7 ± 16.3	0.6
basal ganglia	100.0	100.0 ± 1.0	100.0 ± 1.4	100.0	1.5
hippocampus	47.2 ± 15.8	27.3 ± 5.2	53.0 ± 8.2	37.7 ± 4.8	0.8
occipital cortex	10.1 ± 7.5	13.2 ± 2.8	35.2 ± 6.5	22.1 ± 3.0	0.6
parietal cortex	46.0 ± 17.9	35.5 ± 8.7	53.6 ± 3.7	42.8 ± 6.5	1.0
frontal cortex	98.8 ± 16.7	93.8 ± 14.4	96.5 ± 6.9	94.8 ± 11.2	1.4
olfactory bulb	38.3 ± 7.4	46.4 ± 4.7	60.8 ± 13.6	52.2 ± 4.9	1.1
correlation coeff to band 197/198, *r*		0.98	0.29	0.96	

[a] Values are normalized means ± SEM for three separate experiments. Autoradiographs were scanned and the densitometry data normalized by setting the value for basal ganglia to 100%. The D2$_{A444:415}$ ratio represents the actual, rather than normalized, proportions of the bands. The D2$_A$ column represents values obtained with the 197/198 primer set. D2$_{A444}$ and D2$_{A415}$ values were obtained with the 224/232 primer set.

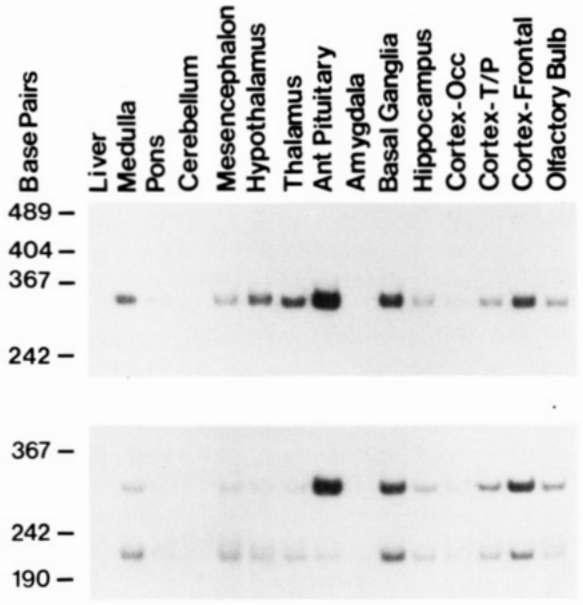

FIGURE 4: Tissue distribution of $D2_A$ alternative transcripts. Total RNA was prepared as described, and equivalent amounts of RNA from each tissue were reverse transcribed with or$D2_A$-224 and -198 primers. 250 ng of cDNA was subsequently amplified with either primer set or$D2_A$-197/198 or primer set or$D2_A$-232/224. (Upper) Reaction products observed with 197/198 primer set. (Lower) Fragments amplified with 232/224 oligonucleotides.

for the presence and relative abundance of the alternatively spliced transcripts. Total RNA from specific brain regions was reverse transcribed with both the complementary exon 3 specific primer (or$D2_A$-198) and the exon 6 primer (or$D2_A$-224). Following second-strand synthesis and DNA amplification, products from both sets of reactions were resolved on acrylamide gels.

Using Northern blots, Bunzow et al. (1988) observed $D2_A$ mRNA predominantly in basal ganglia and anterior and intermediate lobes of the pituitary. Significantly lower amounts of mRNA were observed in other tissues including olfactory bulb, hypothalamus, amygdala, septum, etc. Using the PCR approach, we can confirm, extend, and quantitate these results (Figure 4). Both alternatively spliced transcripts were present in every tissue that expresses $D2_A$ mRNA. The only tissue in which we could not detect $D2_A$ gene transcription was the liver. With very long autoradiographic exposures we detected very low levels of the $D2_A$ alternative transcripts in cerebellum, amygdala, hippocampus, and occipital cortex. The tissue distribution of $D2_A$ mRNA was the same for two different sets of PCR primers (Table III), demonstrating that the observed tissue differences were not secondary to priming artifacts. The overall linear correlation for results for the two primer pairs was 0.96 ($p < 0.001$).

The relative proportions of the two alternative transcripts varied 25-fold across the tissues surveyed (Table III). In 10 of the 14 brain tissues $D2_{A415}$ mRNA predominated. However, in tissues where D2 receptor number is highest, basal ganglia, anterior pituitary, and olfactory bulb, the $D2_{A444}$ mRNA predominated. In fact, the correlation of expression between different PCR primer pairs was much better for the $D2_{A444}$ mRNA ($r = 0.98$, $p < 0.001$) than for the $D2_{A415}$ mRNA ($r = 0.29$, $p > 0.2$). These findings imply the presence of tissue-specific factors that control not only $D2_A$ gene transcription but also alternative splicing of the pre-mRNA.

Many studies have implicated D2 receptors in the development of psychiatric and neurological disorders. Additional studies have demonstrated that chronic drug treatment with D2 receptor agonists can lead to a variety of dyskinetic and cognitive problems. Knowledge of the genetic organization and expression of these pivotal receptors provides the framework from which to conduct a detailed examination of factors involved in their regulation. Hopefully, these studies will lead to a better understanding of the etiology of some of these disorders and to more specific and selective therapies.

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