ARPP-21, A Cyclic AMP-Regulated Phosphoprotein Enriched in Dopamine-Innervated Brain Regions. II. Molecular Cloning and Nucleotide Sequence

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A cDNA clone for the mRNA of bovine ARPP-21 (cAMP-regulated phosphoprotein, $M_r = 21,000$ as determined by SDS-PAGE) was isolated from a modified Okayama-Berg plasmid library. Transformed *Escherichia coli* colonies were screened by *in situ* colony hybridization with 2 different oligonucleotide probes derived from the amino acid sequence of the bovine protein. Sequence analysis of the longest cDNA clone, pTKAI [2407 nucleotides plus a poly(A) tail], revealed a 267-nucleotide-long coding region in agreement with the bovine ARPP-21 amino acid sequence (Williams et al., 1989). Southern blot analysis of total bovine genomic DNA raised the possibility that there may be 2 genes coding for ARPP-21. Northern blot analysis of total cellular RNA from bovine caudate nucleus and other brain regions demonstrated the existence of 2 major mRNA species, 2.5 and 1.0 kb in length, probably derived from use of alternate polyadenylation sites. There was a differential expression of these 2 mRNAs within the brain. Both ARPP-21 mRNAs were most abundant in the caudate nucleus, where the concentration of the protein is highly enriched.

Studies of the regional distribution of substrates for cAMP-dependent protein kinase in mammalian brain revealed that 7 of these proteins are highly enriched in the basal ganglia (Walaas et al., 1983). ARPP-21 (cAMP-regulated phosphoprotein, $M_r = 21,000$ as determined by SDS-PAGE), one of these proteins, has been purified to homogeneity from bovine caudate nucleus, biochemically characterized (Hemmings and Greengard, 1989) and its amino acid sequence determined (Williams et al., 1989). In this paper, we describe the isolation and characterization of a cDNA clone for bovine ARPP-21 identified by use of an *in situ* colony hybridization method with oligonucleotide probes. We have used this clone to study the regional distribution of ARPP-21 mRNA in bovine brain and to carry out a genomic analysis of this protein. The availability of cDNA probes will greatly facilitate the study of the phosphoproteins enriched in the medium spiny neurons of the caudate nucleus, including the regulation of their coordinated expression in this

specific type of neuron. This information should increase the understanding of the role of these proteins in the functions of the medium spiny neurons.

Materials and Methods

Bacterial strains and plasmids. *Escherichia coli* K-12 strains used were WA802 [*metB1*, *lacY* or *lac3*, *galK2*, *galT22*, λ^- , *supE44*, *endA*, *sbcB*, *hsd3*] (Wood, 1966; Nelson et al., 1981), JM103 [Δ (*lac-pro*), *supE*, *thi*, *strA*, *sbcB15*, *endA*, *hspR4*, *F' traD36*, *proAB*, *lacI^qZ Δ M15*] (Messing et al., 1983) and JM109 [*recA1*, *endA1*, *gyrA96*, *thi*, *hsdR17*, *supE44*, λ^- , Δ (*lac-proAB*), *F' traD36*, *proAB*, *lacI^qZ Δ M15*] (Yanish-Perron et al., 1985). Plasmids included pSV7186 (Pharmacia), pGEM4 (Promega Biotec), and pTK501 (Kurihara et al., 1988). *E. coli* strains harboring these plasmids were grown in LB medium containing 50 μ g/ml ampicillin.

Preparation of bovine caudate RNA. Total RNA (2.8 mg) was isolated from calf caudate nuclei (6 gm) by extraction with guanidine isothiocyanate followed by sedimentation through cesium chloride (Chirgwin et al., 1979). Poly(A)⁺ RNA (200 μ g) was enriched by a single cycle of oligo(dT)-cellulose (Type 3, Collaborative Research) chromatography (Aviv and Leder, 1972; Zelenka and Piatigorsky, 1974).

Preparation of oligonucleotide hybridization probes. Two oligonucleotide probes, oligo TK3 and oligo TK4 (Fig. 1), were prepared with the DNA Synthesizer 308A (Applied Biosystems) and purified by Sephadex G-100 (Pharmacia) column chromatography in TEAB buffer (10 mM triethylamine bicarbonate, pH 8.5). The degeneracy of oligo TK3 was 32, and that of oligo TK4 was 1152. Oligonucleotides were labeled at the 5' end with [γ -³²P]ATP using T4 polynucleotide kinase, followed by purification using Sephadex G-50 (Pharmacia) column chromatography. The specific activities of these probes were $>10^8$ cpm/ μ g DNA.

Colony hybridization. A bovine caudate nucleus cDNA library (Kurihara et al., 1988) was screened at a density of 2200 and 6600 colonies/82 mm Petri dish. The DNA from lysed bacteria was transferred to nitrocellulose filters as described by Maniatis et al. (1982) and Grunstein and Hogness (1975) with minor modifications (Kurihara et al., 1988). The conditions for probing the filters have been previously described (Kurihara et al., 1988).

Sequencing of cDNA fragments. The entire cDNA region of pTKAI was inserted into the *Hinc* II site of M13mp19 in both orientations. Sequential DNA deletions were obtained with T4 DNA polymerase (Dale et al., 1985), and sequences were determined by the dideoxy chain termination method (Sanger et al., 1977; Messing, 1983). Sequencing was also performed using restriction fragments of pTKAI inserted into appropriate sites in M13mp18 and mp19 phage (Pharmacia).

Preparation of antisense RNA probes. ARPP-21 antisense RNA probes were used for Northern and Southern blot hybridizations. The antisense probes were synthesized with SP6-polymerase in pGEM4 (Promega Biotec) according to Melton et al. (1984). Plasmids included pTKAI4.2, which contained the 0.65 kb *Pst* I/*Eco*RI fragment with the coding region for ARPP-21, and pTKAI4.3, which contained the 0.45 kb *Pst* I/Kpn I fragment from the 3' noncoding region.

Southern blot analysis. Bovine genomic DNA was cleaved with individual restriction endonucleases and electrophoresed on a 1% agarose gel and transferred to nitrocellulose (Southern, 1975). The filters were hybridized with ³²P-labeled antisense RNA in a mixture as described

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Amino acid	N	Pro	Glu	Gln	Glu	Thr	Ala	Thr	Pro	Glu	Asp	Gly	C
											(Asn)		
Possible codons	3'	<u>GGA</u>	<u>CTC</u>	<u>GTC</u>	<u>CTC</u>	<u>TGC</u>	<u>CGG</u>	<u>TGA</u>	<u>GGC</u>	<u>CTC</u>	<u>CTA</u>	<u>CCc</u>	5'
(OLIGO PROBE TK4)		T	t	t	t	A	A	T	T	t	G	t	
(Actual seq)		C				G	C	C	A	(<u>T</u>)		a	
		G				t	t	G	G			g	

Amino acid	N	Leu	Asp	Glu	Glu	Glu	Lys	Leu	Glu	Leu	Gln	Arg	C
Possible codons	3'	<u>GAC</u>	<u>CTG</u>	<u>CTC</u>	<u>CTC</u>	<u>CTC</u>	<u>TTC</u>	<u>GAC</u>	<u>CTC</u>	<u>GAC</u>	<u>GTC</u>	<u>TCc</u>	5'
(OLIGO PROBE TK3)		a	G	A	t	t	t	a	G	t	a	G	t
(Actual seq)		t						t		t		a	
		a						a		a		g	

Figure 1. Synthetic oligonucleotide probes used for screening of bovine ARPP-21 clones. The possible sense sequences were deduced from the amino acid sequences (Williams et al., 1989) of purified bovine ARPP-21. Codons in capitals were used as probes. The actual sequence as derived from the cDNA pTKA1 is *double underlined*.

by Kurihara et al. (1988) at 65°C with 200 µg/ml yeast tRNA and 100 µg/ml salmon testis DNA for 20 hr. The filters were washed at 65°C in 0.1% SDS, 0.2 × SSC (1 × SSC = 0.15 M NaCl; 0.015 M Na₃ citrate), after a wash in 2 × SSC with 1 µg/ml RNase A at room temperature for 5 min.

Northern blot analysis. Northern blot analysis was performed essentially according to Maniatis et al. (1982) and Thomas (1980). Hybridization procedures were the same as described for Southern blotting, except that the nitrocellulose filters were incubated in a buffer containing 50% formamide at 55°C, with 200 µg/ml yeast tRNA and 100 µg/ml salmon testis DNA, and the filters were washed at 65°C in 0.1% SDS, 0.2 × SSC without formamide. A wash in 2 × SSC with 1 µg/ml RNase A was performed at room temperature prior to the final wash at 60°C in 0.1% SDS, 0.1 × SSC. Calf liver 28S rRNA (4.7 kb) and 18S rRNA (1.9 kb) were used as standards (Pharmacia).

Enzymes and reagents. *E. coli* DNA polymerase I (Klenow fragment), ribo-, deoxy-, and dideoxy-nucleotide triphosphates, *Hind* III linker, and a DNA size marker (DRigest III) were purchased from Pharmacia. RNasin was purchased from Promega. T4 DNA ligase, terminal deoxytransferase, and *E. coli* polynucleotide kinase and restriction endonucleases were purchased from Boehringer Mannheim, Bethesda Research Laboratories, New England Biolabs, or Pharmacia. γ-³²P-ATP (3000 Ci/mmol), [α-³²P]CTP (800 Ci/mmol), [α-³⁵S]ATP (500 Ci/mmol), and [α-³⁵P]CTP (3000 Ci/mmol) were obtained from New England Nuclear. Other chemicals were obtained from standard commercial suppliers.

Results

Isolation of a cDNA clone for ARPP-21 mRNA

Starting with 0.5 µg of poly(A)⁺ RNA from bovine caudate nucleus, a cDNA library of 1.0 × 10⁵ clones was constructed using the Okayama-Berg procedure, amplified, and stocked at -20°C (Okayama and Berg, 1982; Kurihara et al., 1988). This library was screened by the colony hybridization method (Grunstein and Hogness, 1975; Maniatis et al., 1982) using a mix of 2 different oligonucleotide probes (oligo TK3 and oligo TK4). These oligonucleotides were designed on the basis of the amino acid sequence of bovine ARPP-21 (Williams et al., 1989) and mammalian codon usage data (Grantham et al., 1981). The amino acid sequences and the corresponding oligonucleotide probes are summarized in Figure 1.

Out of approximately 2.4 × 10⁵ recombinant colonies, 6 colonies gave positive signals and were independently detected with each of the probes. Restriction enzyme analyses and Southern hybridization revealed that the clones were identical except for variations in length at the 5' end. All further analyses were performed on pTKA1, the longest clone.

Figure 2. Restriction map and sequencing strategy of pTKA1. The scale above the restriction map indicates the nucleotide positions in kilobases (kb) relative to the first nucleotide. The *open box* depicts the coding region for the ARPP-21 protein, while the *thick black line* depicts the untranslated 5' and 3' ends. ATG and TGA represent the start and stop codons, respectively. The *arrows* indicate the sequencing strategy after collecting deletion series of single-strand subclones (Dale et al., 1985). Overlapping sequences in both directions, not shown in this figure, were derived from bacteriophage M13 subclones as templates (Messing, 1983).

TTAATAAATCGACCCAAATAATAACAATAAAGCCAAAGCAAACAAATACAGCCAAAGTCATCCGTGAGAAACAATAATACTAATAAA
 10 20 30 40 50 60 70 80 90
 AGCCACAACCAAAATCAATCAAGGAACAGAGACGCTGGGGGACGTCCACTTTTCGGTCAAGGTGGACGATGTGTGACACCGTGAAGG
 100 110 120 130 140 150 160 170 180
 GAATCACATGGAGATGGGCGTTTGAAGTGTAAATGGGACATGGGACTCTCGTTGTCTCTGATCCCACGTGTGGATTTTCCAGCGAAG
 190 200 210 220 230 240 250 260 270
 AAGGACAGAAGCCGCTCCTAAGTTGCCAAGACCGGCAGCCGGACTCTGCACGAAAGGGTGTCAAACCTGTGATTTTAATTGTCATCTG
 280 290 300 310 320 330 340 350 360
 1 10 20
 M S E P G D L S Q T I V E E G G P E Q E T A T P E N G V
 GGAGAATGTCTGAGCCAGGAGACCTGAGTCAGACCATAGTGGAGGAAGGTGGGCTGAGCAGGAGACGGCCACTCCGGAGAAATGGTGTGA
 370 380 390 400 410 420 <TK4> 430 440 450
 30 40 50
 I K S E S L D E E E K L E L Q R R L V A Q N Q E R R K S K S
 TTAATCTGAAAGTCTGGACGAGGAGGAGAACTGGAATTACAGAGCGGCTGGTGGCTCAGAACCAAGAGAGAAGAAAATCCAAGTCAG
 460 470 <TK3> 480 490 500 510 520 530 540
 60 70 80
 G A G K G K L T R S L A V C E E S S A R P G G E S L Q D Q T
 GAGCAGGAAAAGGTAAATTGACTCGCAGCCTTGTCTGTGAAGAGTCTCGCCAGACCAGGAGGGGAAAGTCTTCAGGACCAGACCC
 550 560 570 580 590 600 610 620 630
 L *
 TCTGAATACTGCACATGGAAGGAATTCAAAAGAATTTAGATTAAATGTAAGCACGGTCGTGTGCTATTCTCTCAAAGGCTCTCTCTTGA
 640 650 EcoRI 660 670 680 690 700 710 720
 TCAGACTGAACCAATACAGTCTTAAGTACCACGTCTCTCCCTTAGTGGAGAGTCTTGCACCTTGCCCTATAAAAAACAAATTTTGGCTC
 730 740 750 760 770 780 790 800
 TTAGGAAGTTTCAGAAATAGTGAATAAGGTGCATTGAGCTTTGGACACATACCTTTAGGGCCTTTGGTGGAGATTCTCACTACCGGAAA
 820 830 840 850 860 870 880 890 900
 AATGGTCCAGAAATAAATCTGAGATGAATGTGAATTTAAGTTAATATTATTAATACTTTGCTGCACATCCTTACACCTAATTTTGTCTCC
 910 920 930 940 950 960 970 980 990
 TTACAGCAAGGCTGTTTCAGCTCTAAAAATCAACTTCCAACAAAACCTTTCACATGGTATTTATTTTGGCTTTTCAGGCATTATAT
 1000 1010 1020 1030 1040 1050 1060 1070 BglI 1080
 TCGCTTTTAGAAAAATGGTCTTGTCTGTTAAATTTTCTCTGTTTATCAGAGGCTGAGAACAGTCAGGACAAAAGTAAATGATTGATC
 1090 1100 1110 1120 1130 1140 1150 1160 1170
 TGAAGTCAAGAGGAGGAAAAATTAAGTTAGCATTATTTGAGGGGCTGTGTTTGCCTTTTCTTTATAAATTCATTAAAAATCTCAAAT
 1180 1190 1200 1210 1220 1230 1240 1250 1260
 TTTCTCTTGGATACAGAGAGCGTTGAGAGCACTTTCTTTCAAGGACCAAAAAATAAATTCCTCGTAGATTTCATCCTTAGAGTATTTT
 1270 1280 1290 1300 1310 1320 1330 1340 1350
 TTCCCCCAGCCTCATTTCCTCAACCTGCCACCCATGTCGTACGCCAAGGGCGAGCTAGCTTTCAGTAGCCATCACTATGTTTATACAAG
 1360 1370 1380 1390 1400 1410 1420 1430 1440
 TTTTATTTTCTTGGGCTCTGAGAAACGTGTGGCTTTTGTTCAGCATTATTTATTTGTGCTTCTTTTGTGTTGGAGATTAAAGGGGGAA
 1450 1460 1470 1480 1490 1500 1510 1520 1530
 TGAGGCGAATATCACAGCTTATATCATTTCAGTGTGGATGGATGGCTTGTGATGTAATGCACACAATACATGTTAATTCTGCAGAAATGACA
 1540 1550 1560 1570 1580 1590 1600 1610 PstI 1620
 GACTCTGAGAGAAATAATGCCAGTGTCCCTACTCCGAATGCCAGGCAGAGAACAACAGCCTTTATAGATTTTCATCTGATTTTATTA
 1630 1640 1650 1660 1670 1680 1690 1700 1710
 ATCCTATTTGATGAGGTACTGGGAGGAGGTTTTTTTCGAGTGATCATCTGTATCATTGTACCCACCCTGGACCTAAGACTAGGTTACTC
 1720 1730 1740 1750 1760 1770 1780 1790 1800
 CTTGCAACTGGAGCAGAGAAATAAGAGGCAATGGTACTTTGCTCCTGCTCGCCCATGCTGAGTAACCTTGAGACTAGCTTTGTAGGAAT
 1810 1820 1830 1840 1850 1860 1870 1880 1890
 TAGAAGTTCGAAGCCTTCCTTGGGACTGGCTGAGGAGTGGGAGCAGGGCCCGCCCCCACCACCCCTCACACATATAAGCAAGTGGC
 1900 1910 1920 1930 1940 1950 1960 1970 1980
 CATGCTGCTCTTTTAGAGAATAAAGAAGCAGACCTTTGAGCTTGATCCAATGCCTGCATTAGGTACCACCGGCACTTAACAACATGTGG
 1990 2000 2010 2020 2030 2040 KpnI 2050 2060 2070
 GAACTGAATCCCCGACAACACTGGCCGAAGTGTACGGCATACGGATTGTGTGGCTCCCCCAATGTGTGTGATTACAGTATTTGGAAAA
 2080 2090 2100 2110 2120 2130 2140 2150 2160
 CTGCCTTCATTTTCTGTGTGGGAAAAAATTTTGCTACCTGTATTACTTGTATCTCAGACCCATAGCAGATGGTTAGCTGTCTCTTAAG
 2170 2180 2190 2200 2210 2220 2230 2240 2250
 TTAAAGGAAGTGTCTTTCTTAATGTTATACTCTTTACCTATCAGTGTATTGTGCAACTTGAATCATTTCTTTCTGTTGTTGGAGA
 2260 2270 2280 2290 2300 2310 2320 2330 2340
 TAACGTTATCCTGTACTGTATTTAAACACTGTATTATTATTGAGCATATCAATAAAAAATATTAn
 2350 2360 2370 2380 2390 2400

Figure 3. Complete nucleotide sequence of pTKAl and the derived amino acid sequence of bovine ARPP-21. Nucleotide numbering begins at 1 for the first nucleotide after the G tail in the vector. The areas used to generate the synthetic oligonucleotide probes are underlined and labeled <Tk3> and <Tk4>. Restriction sites are underlined (—), and potential polyadenylation sites are double underlined (==).

Identification of ARPP-21 cDNA by nucleotide sequencing

A restriction map analysis and the sequencing strategy for pTKAl are presented in Figure 2. The complete base sequence and deduced amino acid sequence of clone pTKAl are shown in Figure

3. The sequence data indicate that pTKAl is 2407 nucleotides in length, excluding the poly(A) tail. It contains the complete coding sequence corresponding to the bovine ARPP-21 protein, coding for 89 amino acids, including the initiation codon ATG, as well as the termination codon TGA. There are 365 nucleo-

Figure 4. Southern blot analysis of bovine thymus genomic DNA. Twenty micrograms of bovine thymus DNA were digested with restriction endonucleases, electrophoresed through a 1% agarose gel, transferred to a nitrocellulose filter, and hybridized with ^{32} P-labeled pTKAIG4.2, containing the ARPP-21 coding region, but excluding the 3' untranslated end. Lane 1, *Eco* RI; lane 2, *Pst* I; lane 3, *Eco* RI and *Pst* I; lane 4, *Kpn* I; lane 5, *Bam* HI; lane 6, *Hind* III. Sizes were calculated from coelectrophoresis of *DR*Igest III (Pharmacia).

tides of 5' noncoding region and 1775 nucleotides of 3' noncoding region. There are 4 potential polyadenylation signals, as the consensus sequence AATAAA (Proudfoot and Brownlee, 1976) is present at positions 912, 1314, 1999, and 2396. The aspartate at position 25 in the amino acid sequence (Williams et al., 1989) is an asparagine coded by AAT in the DNA sequence. [Amino acid positions are numbered according to the protein sequence in the accompanying paper by Williams et al. (1989), with the first serine being in position 1, as the initiator methionine is not present in the amino acid sequence.] The aspartate may be derived from asparagine by deamidation during the protein purification or peptide-sequencing procedure, or from a second coding sequence. It is unlikely to arise from a second coding sequence as all 6 cDNAs coded for an asparagine with AAT in that position.

Southern blot analysis of bovine genomic DNA

In order to investigate the genomic organization of ARPP-21, chromosomal DNA from bovine caudate nucleus was analyzed with a probe derived from pTKAIG4.2 (Fig. 4). There were multiple hybridizing bands in all lanes, including those digested with *Bam* HI and *Hind* III, for which there are no restriction sites in the cDNA. It appears that either the 0.65 kb *Pst* I/*Eco* RI fragment of the cDNA is derived from a minimum of 2 exons, or that there are 2 genes coding for ARPP-21 mRNA. We attempted to answer this question by performing Southern blot analysis with 2 separate oligomers from nucleotide 1–30

or from 624–654. We found that we could not adequately compare the results of a Southern blot hybridized with a 658 ntd RNA probe to one hybridized with a 30 ntd, end-labeled oligomer (data not shown). The definitive answer requires analysis of the entire gene.

Distribution of ARPP-21 mRNA in bovine CNS

The distribution of ARPP-21 mRNA in the bovine CNS was analyzed by Northern blot hybridization with a probe derived from pTKAIG4.2 (Fig. 5). A predominant mRNA species at 2.5 kb was highly enriched in the caudate nucleus, in agreement with the large amount of protein in this region (Walaas et al., 1983; Hemmings and Greengard, 1989; Ouimet et al., 1989). This mRNA species was also relatively abundant in the cortex, particularly in the occipital and motor areas (Fig. 5A). The 2.5 kb band was barely detectable in the hippocampus, cerebellum, or thalamus and was undetectable in the substantia nigra, medullary white matter, and adrenal gland. A minor band at 1.75 kb could only be detected with pTKAIG4.2 in caudate nucleus poly(A)⁺RNA (Fig. 5B).

Hybridization with pTKAIG4.2, which contains the coding region for ARPP-21, also yielded a band at 1.0 kb (Fig. 5). Of note, this was the mRNA species with the greatest amount of hybridization in total cellular RNA from the cerebellum, and also appeared to be present in very small amounts in the substantia nigra. pTKAIG4.3, extending from nucleotide 1613 to

Figure 5. *A*, Regional distribution of ARPP-21 mRNA in bovine nervous tissue by Northern blot analysis. Twenty micrograms of total RNA were separated by electrophoresis in 1.2% agarose gel containing 2.2 M formaldehyde and analyzed by Northern blot hybridization with a ^{32} P-labeled antisense RNA probe derived from pTKAlG4.2. *Lane 1*, caudate nucleus; *lane 2*, substantia nigra; *lane 3*, occipital cortex; *lane 4*, prefrontal cortex; *lane 5*, entorhinal cortex; *lane 6*, motor cortex; *lane 7*, hippocampus; *lane 8*, cerebellum; *lane 9*, thalamus; *lane 10*, medullary white matter; *lane 11*, adrenal gland. *B*, Northern blot analysis was performed as in *A*. *Lane 1*, 10 μ g A⁺ RNA from bovine caudate nucleus; *lane 2*, 10 μ g A⁻ RNA from bovine caudate nucleus.

2047, hybridized only to the 2.5 Kb mRNA species in caudate nucleus RNA and did not hybridize to cerebellar RNA (Fig. 6).

Discussion

In this paper, we describe the cloning of a cDNA for bovine ARPP-21, a neuron-specific phosphoprotein, which is highly enriched in neurons containing the D-1 dopamine receptor, particularly the medium spiny neurons of the caudate nucleus (Ouimet et al., 1989). Our sequence data (Fig. 3) indicate that the longest clone, pTKAl, has 2407 nucleotides, excluding the poly(A) tail. It codes for a protein of 89 amino acids (267 nucleotides), which corresponds to the bovine ARPP-21 protein except for the presence of the initiator methionine and the substitution of asparagine for aspartate at position 25. The possible reasons for these discrepancies are discussed above and in the accompanying paper (Williams et al., 1989). The seryl residue, which is phosphorylated by cAMP-dependent protein kinase, is present at position 55. ARPP-21 contains a high percentage of acidic residues (20%), as does DARPP-32 (Williams et al., 1986; Kurihara et al., 1988), another cAMP-regulated phosphoprotein highly enriched in the medium spiny neurons of the striatum. There is no sequence homology between ARPP-21 and DARPP-32.

Hybridization of bovine caudate poly(A)⁺ RNA with a probe

that included the coding region for ARPP-21 revealed 3 bands, 2 of which were visible after hybridization with caudate nucleus total cellular RNA. The major band at 2.5 kb almost certainly represents the mRNA species from which pTKAl was cloned. The minor band at 1.0 kb can also be clearly detected in total cellular RNA. There are 4 potential polyadenylation signals, as identified by the sequence AATAAA, at positions 912, 1314, 1999, and 2396. The fact that only the 2.5 kb species is detected with a probe derived from the 3' untranslated region from nucleotide 1608 through 2043 strongly suggests that the 2 RNA species differ in use of alternate polyadenylation sites and in the length of the 3' untranslated end. We do not yet know the physiologic significance, if any, of region-specific use of alternate polyadenylation sites. Only extremely low levels of ARPP-21 protein can be detected in the cerebellum, and the protein is located only in terminals in the substantia nigra (Ouimet et al., 1989). There is increasing evidence that the untranslated 3' end of an mRNA species may be important in regulation of mRNA stability and efficiency of translation (Shaw and Kamen, 1986; Strickland et al., 1988), and it is possible that the long 3' untranslated end present in the 2.5 kb mRNA species is required for translation. The 2 mRNA species are unlikely to represent the 2 different forms of ARPP-21 protein (see accompanying paper, Williams et al., 1989) as ARPP-21A and ARPP-21B are

Figure 6. Northern blot analysis of 20 μ g bovine caudate nucleus (lane 1) and cerebellum (lane 2) total cellular RNA with a ^{32} P-labeled antisense RNA probe derived from pTKAIG4.3 (nucleotides 1613–2047). Hybridization and washing conditions were the same as in Figure 5.

present in approximately equal amounts (Hemmings and Greengard, 1989).

Southern blot analysis of bovine thymus genomic DNA revealed that there were multiple restriction fragments that hybridized to ARPP-21 antisense RNA derived from the 5' end of the clone, including the coding region. Multiple bands were present even when the DNA was digested with enzymes for which there are no sites in the entire ARPP-21 cDNA, including *Bam* HI and *Hind* III. There are 2 possible explanations for this finding. The first is that there are, within this 5' portion of the gene, intron(s) that have restriction sites for each of the enzymes used in the analysis. The second is that there are 2 genes coding for ARPP-21. These possibilities will be distinguished via study of the structure of the ARPP-21 gene. If there are 2 ARPP-21 genes, the 2 major mRNA species observed may be derived from separate genes.

The regional distribution of ARPP-21 mRNA correlates with that of the protein as detected by immunoprecipitation (Hemmings and Greengard, 1989; J.-A. Girault, unpublished observations) and immunocytochemistry (Ouimet et al., 1989). The

greatest concentrations are found in the basal ganglia, particularly in the striatum. Significant, but lower, levels are detected in the cortex, and even lower levels are present in several other brain regions. Like DARPP 32, the highest concentrations of ARPP-21 mRNA and protein are present in areas with the greatest density of D-1 receptors (Boyson et al., 1986), particularly the striatum. The absence of the predominant ARPP-21 mRNA species in the substantia nigra is consistent with the fact that, by immunocytochemistry, ARPP-21 is localized to axons and axon terminals, and not to cell bodies in this region (Ouimet et al., 1989).

In conclusion, we present the structure of a cDNA that encodes a neuron-specific phosphoprotein, ARPP-21. Northern blot analysis shows that the distribution of the mRNA correlates with the localization of ARPP-21-containing cell bodies as detected by immunocytochemistry. In addition, there are 2 major mRNA species, probably arising by differential use of alternate polyadenylation sites. Southern blot analysis suggests that either there are multiple introns within the gene or that there are 2 genes of somewhat different structure. Further analysis of the structure of the ARPP-21 gene and regulation of ARPP-21 mRNA will provide a basis for a detailed investigation of the mechanisms of neuron-specific expression of ARPP-21 within the brain.

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