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Molecular characterization of Br-cadherin, a developmentally regulated, brain-specific cadherin

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ABSTRACT **Cadherins are a family of transmembrane proteins that play a crucial role in cell adhesion and in morphogenesis. Several of the cadherins are expressed in the nervous system, but none is neuron-specific. We characterize a new member of the cadherin family, Br-cadherin, which is present exclusively in the central nervous system. Although the Br-cadherin protein is confined to the central nervous system, its mRNA is present in several additional tissues, suggesting that there is posttranscriptional control of this gene's expression. Within the central nervous system, Br-cadherin appears to be expressed specifically by neurons. In the mouse, its expression becomes detectable during the first postnatal week, which corresponds temporally to the onset of synaptogenesis and dendrite outgrowth in the brain. This pattern of expression is consistent with a role for Br-cadherin in neuronal development, perhaps specifically with synaptogenesis.**

Cell adhesion molecules mediate contact-dependent processes that are essential requirements for cell migration and morphogenesis during development. The cadherins, a large family of cell surface molecules, are a well characterized group of transmembrane glycoproteins that function as cell adhesion molecules. Cadherins interact with each other via Ca^{2+}-dependent, homophilic, and, less commonly, heterophilic binding to other cadherin molecules (1, 2), as well as other cell adhesion molecules (3). In addition to having adhesive properties, cadherins are involved in cell signaling by activation of second messenger pathways; there is an accumulating body of evidence that shows this involvement (reviewed in refs. 4 and 5).

Cadherins have a cannonic structure consisting of a long extracellular (EC) domain of five repeats, located at the amino terminus of the protein (2, 6). Conserved motifs among different cadherins in the EC domain include putative glycosylation and calcium-binding sites. A cell adhesion recognition sequence, which is thought to facilitate binding, is present in the first EC repeat. After the repeats, the majority of cadherins have a single transmembrane domain and a short and highly conserved cytoplasmic domain that associates indirectly with the actin cytoskeleton via the catenin and α-actinin proteins (7–9).

Most cadherins are expressed both during embryonic development and in the mature organism (reviewed in refs. 4 and 9). The critical role that cadherins play in neuronal development has been repeatedly demonstrated. Neurulation, neuroepithelial development, and neurite outgrowth depend on the presence of cadherins (2, 6), and disturbance in their expression results in grossly abnormal development of the nervous system (10, 11). For example, injection of antibodies against N-cadherin into chicken embryos results in abnormalities of the neural tube and defective migration of the neural crest (12).

Multiple cadherin genes are expressed in the nervous system (2, 5, 13, 14), but all are expressed in other tissues as well. Here we describe a new member of the cadherin family, Br-cadherin, whose protein is uniquely expressed in the brain. Previously, we cloned a partial cDNA of Br-cadherin as part of an effort to identify brain-derived transcripts from the spinal muscular atrophy region on human chromosome 5q13 (15, 16). Further analysis of this cDNA revealed that, although several copies of an expressed Br-cadherin pseudogene are localized to the spinal muscular atrophy region, the full length, intact Br-cadherin gene is located on the opposite arm of chromosome 5, at 5p13–14 (17). A partial sequence of the gene (designated as cadherin-12) was described by Tanihara *et al.* (18).

The development course of Br-cadherin expression also is distinctive. Unlike other cadherins, Br-cadherin is detected only postnatally in the mouse, and its expression increases gradually during the first week of life to adult levels. The onset of expression in the mouse correlates with simultaneous increasing neurite outgrowth and synaptogenesis; thus, Br-cadherin is temporally and spatially well localized to play a role in a critical period in neurogenesis.

MATERIALS AND METHODS

DNA Sequencing and Intron/Exon Border Analysis. Genomic phages encompassing the human Br-cadherin locus were cloned as described (17). Exon-containing restriction fragments from these phages were detected by hybridization to Br-cadherin cDNA. These fragments were subcloned into pBluescript II SK(+) plasmid vectors (Stratagene) and sequenced with primers based on the cDNA sequence. Sequencing was performed with an Applied Biosystems sequencer using *Taq* DNA polymerase cycle sequencing, and acquired data were analyzed using sequencher software (Genecodes, Ann Arbor, MI). To determine intron/exon borders, the Br-cadherin cDNA sequence was compared with the genomic sequences by the GAP function of Genetics Computer Group (Madison, WI) software. The presence of consensus splicing signals at points of sequence divergence was identified by direct inspection.

Intron Size Determination. Intron sizes were determined by PCR amplification of total human DNA or genomic phage DNA using cDNA primers situated in close proximity to intron/exon borders. For introns larger than 5 kb, TaKaRa Ex

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Abbreviations: EC, extracellular; UTR, untranslated region; anti-Br-cad-EC1, antibodies for human Br-cadherin.

Data deposition: The additional information on the intron/exon borders has been deposited in the GenBank database (accession no. L33477).

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Taq polymerase (Takara Shuzo, Kyoto) was used with extension times of 7–10 min at 72°C for 30 cycles. PCR products were separated by electrophoresis on 0.4% agarose gels along with high molecular markers (GIBCO/BRL).

Northern Blot Analysis. Northern blot analysis and 5′-untranslated region (UTR) probe preparation were done as described (17).

Antibody Production. Antibodies for human Br-cadherin (anti-Br-cad-EC1) were generated against the peptide CPQYVGKLHSDLDKG from the amino terminus of the Br-cadherin protein (amino acids 72–85). The C residue, which is not present in Br-cadherin, was added to the amino terminus of the peptide as a linker for use in affinity purification. The peptide was synthesized, purified, coupled to keyhole limpet hemocyanin, and used to immunize New Zealand rabbits (Quality Control Biochemicals, Hopkington, MA). Crude antiserum, when used at a 1:5000 dilution, was adequately specific for immunoblotting; a single band was detected in whole cell extracts from human, mouse, and rat brains, and preimmune serum failed to detect a similar band.

Protein Expression in Bacteria. A fusion protein pGEX-EC1 was expressed from a pGEX-4T1 plasmid (Pharmacia) containing the sequence coding the first EC repeat (amino acids 55–157) of Br-cadherin. This construct included the peptide sequence used to generate the antibody. The expected molecular mass of the fusion protein is ≈37 kDa (including the 26-kDa glutathione *S*-transferase protein). The recombinant plasmid was transformed into XL1-blue bacteria (Stratagene), and readily detectable amounts of fusion protein were produced in these bacteria without isopropyl β-D-thiogalactoside induction. The bacteria were lysed in sample buffer [0.0625 M Tris, pH 6.8/10% glycerin (vol/vol)/2% SDS (wt/vol)/10 mM EDTA/50 mM DTT/0.29 M α-mercaptoethanol/5 mg/ml leupeptin/1 mg/ml pepstatin/40 mg/ml phenylmethylsulfonyl fluoride/1.5 mg/ml aprotinin/0.2 mM iodoacetamide/0.8 mM benzamidine] and boiled for 5 min, and cell debris was removed by centrifugation.

SDS/PAGE and Immunoblots. Tissues were frozen in liquid nitrogen, pulverized, and added to the sample buffer described above, boiled for 5 min, sonicated for 45 s, and centrifuged for 5 min to remove debris. Tissue culture protein extracts were prepared by washing the dishes with PBS, adding sample buffer, scraping the dish with a plastic cell scraper, collecting the lysed cells, boiling, sonicating, and centrifuging to remove debris. Electrophoresis of samples was carried out in SDS/polyacrylamide gels (19) using 7.5% resolving gels and 5% stacking gels. Sample loading was equalized according to staining with Coomassie blue. Immunoblotting was performed as described (20). After transfer, filters were stained with ponceau S solution (Sigma) to check protein loading and were blocked in 5% nonfat dry milk in TBST (10 mM Tris, pH 8.0/0.1% Tween 20/150 mM NaCl) for 30 min. All primary and secondary antibodies were diluted in TBST as were all washes. Blots were incubated with first antibody for 14 h at 4°C, washed three times for 15 min, blocked in 5% dry milk for 10 min, incubated with secondary antibody for 1 h, and washed again three times for 15 min. Signals were detected by chemiluminescence using an enhanced chemiluminescence kit (Amersham) according to the manufacturer's instructions. Luminescence was detected by exposing the filter to film for time intervals of 0.5–5 min. The dilutions of primary antibodies were done as follows: anti-Br-cad-EC1, 1:5000; anti-CaM-kinase II (Boehringer Mannheim), 1:500; anti-microtubule-associated protein 2 (Sigma), 1:2000; and anti-synaptophysin (Sigma), 1:1000. Dilutions of secondary antibodies were as follows: donkey anti-rabbit horseradish peroxidase (Amersham), 1:15,000; and sheep anti-mouse horseradish peroxidase (Amersham), 1:10,000.

Glial and Neuronal/Glial Cultures. Rat glial cultures were prepared as described (21). Rat neuronal cultures were prepared from hippocampi of P0 rats and were grown on glial feeder layers. This procedure was done as described (22) except that the growth medium did not contain kynurenic acid but contained a normal Mg^{2+} concentration (1 mM). The cultures were irradiated 1 day after neuron plating and were harvested 5 days after plating.

RESULTS

Genomic Organization of Br-Cadherin. The intron/exon borders were determined by analysis of genomic phages isolated by hybridization to Br-cadherin cDNA (Table 1). Subcloned exon-containing fragments were sequenced using primers derived from cDNA sequences. Distances between many of the exons were determined by PCR amplification from either phage or genomic DNA templates (Fig. 1). Some introns could not be amplified by PCR using exonic primers, presumably because of lengths beyond the range of PCR amplification. The maximum size amplified by TaKaRa Ex *Taq* polymerase was 15.8 kb, and this implies that the introns that were not amplified were greater than ≈15 kb. Taking this in account, the minimum genomic size of the gene is 120 kb. The size of the Br-cadherin gene is comparable to other cadherin genes that might span genomic regions of 200 kb or more (23, 24). Br-cadherin has an unusually long 5′-UTR encoded by five separate exons, with the ORF beginning in exon 5. The 5′-UTR contains 13 alternative AUG codons that potentially could initiate translation of peptides up to 59 amino acids long, but only the one that is positioned at base pair 1089 (sequence accession no. L33477) initiated translation of a protein with structural similarity to other cadherins.

Table 1. Intron/exon boundaries of Br-cadherin

Exon	Exon		Exon size, bp
1		CATCAG	567
		567	
2	CTTTGT	TTAAAG	94
	568	661	
3	CTTTGA	TAACTT	95
	662	756	
4	ACAGTG	ATTAAG	146
	757	902	
5	GCCCCT	GGAAAG	417
	903	1319	
6	CTCCAT	CTGTGG	295
	1320	1614	
7	GTGCAT	AGACAG	120
	1615	1734	
8	GTGTTA	CCAAAA	168
	1735	1902	
9	GCATCT	AAAAAG	188
	1903	2090	
10	CCTTTA	TGTTAG	254
	2091	2344	
11	GTACTT	AAGTTA	137
	2345	2481	
12	GTAACC	GGACAG	122
	2482	2603	
13	ATAATT	TCAGAA	118
	2604	2721	
14	ACAACA	TCTTAG	252
	2722	2973	
15	CCATAG	3′-end	1194
	2974	4167	

cDNA sequences present at the 5′- and 3′-exon ends with the corresponding cDNA base pair number according to the sequence in the GenBank database (accession no. L33477). The sizes of the exons are depicted on the right.

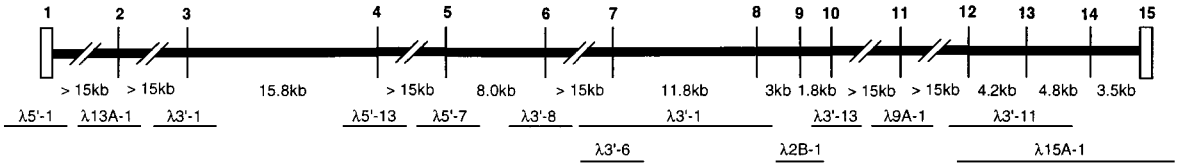

FIG. 1. Genomic organization of the Br-cadherin gene. The 15 exons of Br-cadherin mRNA are depicted by vertical lines and boxes and by the exon number. The genomic phages appear below their corresponding exons. The introns appear as thick bars. Their sizes were determined by PCR. Those that failed to amplify (introns 1, 2, 4, 6, 10, and 11) are shown by interrupted lines, and their predicted size is larger than 15 kb, the size of the largest intron that was amplified by TaKaRa Ex *Taq*.

Tissue- and Developmental-Specific Transcription of Br-Cadherin. Having cloned the Br-cadherin cDNA from human brain cDNA libraries, we screened a broad range of tissues for Br-cadherin mRNA expression, extending our previous study (17). We used a probe from the unique 5′-UTR to minimize the possibility of cross-hybridization with any as yet unidentified homologous cadherins. A consistent 4.4-kb transcript was detected; in some samples, additional bands of 7.5 and <10 kb were present as well (Fig. 2). Our longest characterized cDNA was 4.3 kb, consistent in size with the smallest transcript detected by Northern blots. The 7.5- and <10-kb signals presumably represent partially processed or alternatively spliced mRNAs. Among RNA samples derived from adult humans, the 4.4-kb transcript was detected in testis and in ovary (Fig. 2*a*), in addition to our previous detection of the transcript in adult brain (17). Further analysis of the transcript distribution in brain demonstrated that the transcript is abundant in several gray matter regions (Fig. 2*b*) but not in the corpus callosum (white matter). A similar analysis of multiple fetal tissues reveals Br-cadherin mRNA expression in brain and kidney (Fig. 2*c*) [in adult kidney, no expression was found (17)]. Examining mRNA from various murine tissues indicates that the homologous murine gene is expressed in the adult mouse brain, but, unlike in humans, adult mouse testis shows no expression (Fig. 2*d*). Note that the smallest transcript detected in the mouse was 7.5 kb. We currently are analyzing the murine Br-cadherin cDNA to investigate the possibility of longer UTRs because the length of the ORF in the mouse is identical to that of the human cDNA (S.S., S.A.B., and L.M.K., unpublished data).

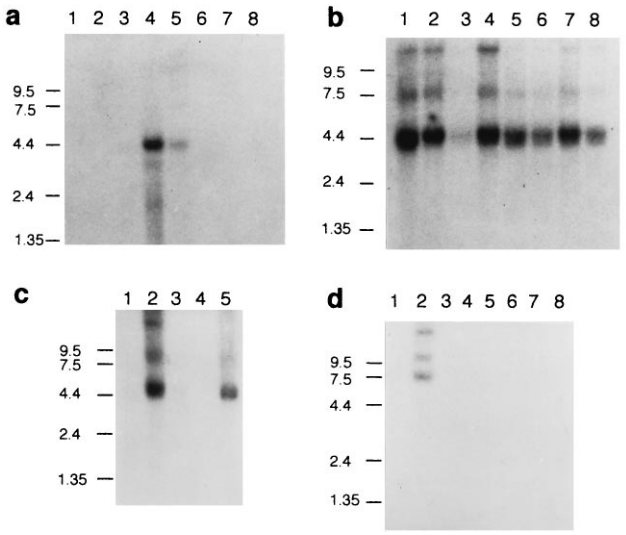

FIG. 2. Tissue distribution of Br-cadherin mRNA. mRNA was analyzed by hybridization to multiple tissue Northern blots (CLONTECH) using a PCR probe from the 5′-UTR of Br-cadherin as described (17). (*a*) mRNA expression in human adult tissues. Lanes: 1, spleen; 2, thymus; 3, prostate; 4, testis; 5, ovary; 6, small intestine; 7, colon; and 8, peripheral blood lymphocytes. (*b*) mRNA expression in different regions of human adult brain. Lanes: 1, amygdala; 2, caudate nucleus; 3, corpus callosum; 4, hippocampus; 5, hypothalamus; 6, substantia nigra; 7, subthalamic nucleus; and 8, thalamus. (*c*) mRNA expression in human fetal tissues. Lanes: 1, heart; 2, brain; 3, lung; 4, liver; and 5, kidney. (*d*) mRNA expression in mouse adult tissues. Lanes: 1, heart; 2, brain; 3, spleen; 4, lung; 5, liver; 6, skeletal muscle; 7, kidney; and 8, testis. All human expressing tissues detected a 4.4-kb signal. Additional signals of 7.5 kb and a signal larger than 9.5 kb were detected in various brain regions and in human fetal brain. The human probe cross-hybridized with rodent brain RNA and detected three different sized transcripts, as well. Size markers are indicated in kilobases.

Preparation of Polyclonal Antibodies to Br-Cadherin. A peptide corresponding to a domain from the first EC repeat of Br-cadherin served as an antigen to generate polyclonal antibodies to Br-cadherin (anti-Br-cad-EC1). An *Escherichia coli* fusion protein, pGEX-EC1, that contains the first EC repeat of Br-cadherin was prepared to test the antisera. Bacterial extracts were immunoblotted and reacted with the serum (Fig. 3*a*, lanes 1 and 2). The anti-serum detects a specific band at the predicted molecular mass of 37 kDa in the *E. coli* extracts containing the fusion protein but not in *E. coli* extracts containing only the original glutathione *S*-transferase protein. The calculated molecular mass of Br-cadherin from its conceptual translation was 82 kDa although a larger actual molecular mass might be expected because cadherins are glycosylated. On examination of human brain extracts with anti-Br-cad-EC1, a single 150-kDa band was detected that was not detected with preimmune serum (Fig. 3*a*, lanes 3 and 4). The fact that anti-Br-cad-EC1 detects the fusion peptide and recognizes a single-sized protein on Western blots supports the specificity of our antibody to Br-cadherin. The amino acid sequence of the peptide chosen to generate the anti-Br-cad-EC1 antibodies was found to be completely identical in murine Br-cadherin (results not shown), and a protein of 150 kDa was detected by Western blot analysis of murine tissues as well.

Br-Cadherin Protein Distribution in Human and Murine Tissues. To compare Br-cadherin protein distribution to the RNA distribution, an immunoblot of a variety of human tissues was prepared, and Br-cadherin protein was detected solely in the brain (Fig. 3*b*). Although the Northern blotting analysis detected abundant Br-cadherin mRNA in human fetal brain (Fig. 2*c*), relatively low levels of protein were present by immunoblotting of this tissue. Disparities between mRNA and protein expression also were found in human adult testis and human fetal kidney, which demonstrated high levels of mRNA (Fig. 2 *a* and *c*) and no detectable protein (Fig. 3*b*). Additional human fetal kidney cell lines were examined by Western blotting, and no Br-cadherin protein was detected (results not shown). The absence of protein in tissues in which mRNA was detected suggests the possibility of posttranscriptional regulation. In a survey of murine tissues, Br-cadherin protein was detected only in adult brain tissues (Fig. 3*c*). The distribution in the brain is widespread, which was demonstrated by dissecting different regions of murine brain and immunoblotting with anti-Br-cad-EC1 (Fig. 3*d*). One intriguing species difference is that Br-cadherin was present in readily detectable quantities in murine cerebellum but not in human cerebellum (Fig. 3 *b* and *d*). The tissue distribution of Br-cadherin as detected by anti-Br-cad-EC1 further supported the specificity

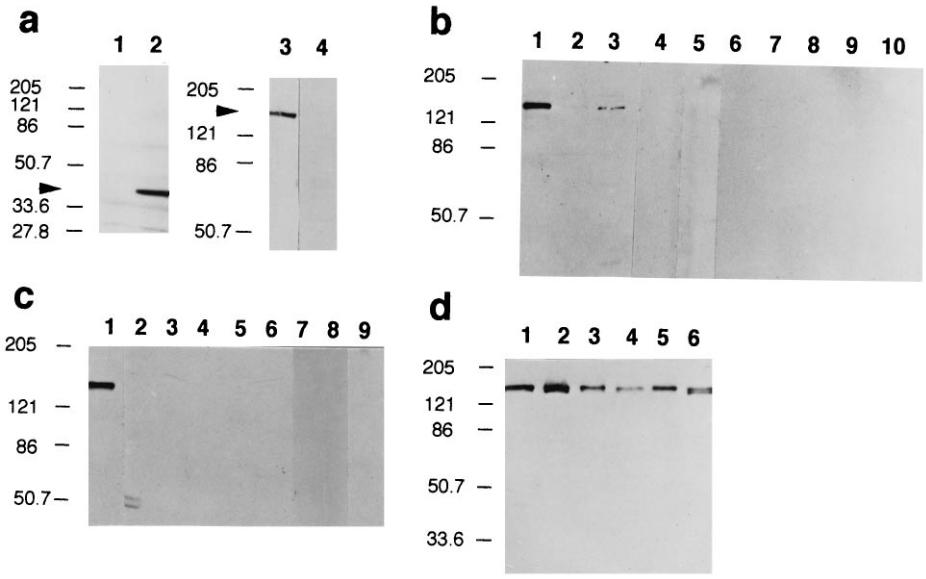

FIG. 3. Tissue distribution of Br-cadherin protein as detected with anti-Br-cad-EC1. (*a*) Anti-Br-cad-EC1 detects a Br-cadherin fusion protein, pGEX-EC1 (lane 2), but does not detect such a protein in extracts that contain the glutathione *S*-transferase protein alone (lane 1). The arrowhead on the left points to the estimated size of the fusion protein (37 kDa). Anti-Br-cad-EC1 recognizes a 150-kDa protein in a human brain extract (lane 3). Preimmune serum does not detect a similar-sized band (lane 4). (*b*) Br-cadherin protein distribution in human tissues as detected by anti-Br-cad-EC1. Extracts from multiple human adults tissues and two fetal human tissues (brain and kidney) were examined for presence of Br-cadherin protein. Lanes from left to right: 1, adult cerebral cortex; 2, adult cerebellum; 3, fetal brain; 4, adult peripheral nerve; 5, adult skeletal muscle; 6, adult testis; 7, adult pancreas; 8, adult liver; 9, adult kidney; and 10, fetal kidney. (*c*) Br-cadherin protein distribution in adult mouse tissues. Lanes from left to right: 1, forebrain; 2, cardiac muscle; 3, lung; 4, kidney; 5, liver; 6, spleen; 7, heart; 8, intestine; and 9, testis. (*d*) Br-cadherin distribution in multiple regions in mouse brain. Lanes from left to right: 1, forebrain; 2, cerebellum; 3, hippocampus; 4, thalamus; 5, striatum; and 6, brainstem. Size markers are indicated in kilodaltons.

of the antibody because none of the characterized cadherins shows a similar protein distribution. This includes rat K-cadherin (25), which has a peptide sequence very similar to that used to generate anti-Br-cad-EC1.

Time Course of Br-Cadherin Expression. Cadherins have been implicated in developmental processes and several demonstrate temporally specific expression during embryogenesis (5, 6). Examining this aspect of Br-cadherin expression, we found that Br-cadherin protein was present not only in human adult brain but also in fetal brain at low levels (Fig. 3*b*). To define the time course of Br-cadherin expression, we examined C57BL/6J mice at different fetal stages and throughout the first weeks of life. No Br-cadherin was detected in samples collected between E6 and P0, indicating that Br-cadherin is not expressed during murine fetal development (Fig. 4*a*). However, after birth, the level of Br-cadherin protein raises gradually, and by 2 weeks postnatal the level is identical to that of adult mice (Fig. 4*b*). This time course is seen in samples from both the cerebral cortex and the brainstem. In contrast, protein extracts from the cerebellum only showed a constant low level of Br-cadherin protein throughout the first week of life. At 2 weeks of age, Br-cadherin is detected in the cerebellum at levels similar to those in the forebrain. Data points in the 2nd week of life were not obtained, but presumably Br-cadherin rises gradually during the 2nd week of life in the cerebellum.

Cellular Localization of Br-Cadherin in the Central Nervous System. Northern blot analysis results revealed low levels of expression in white matter represented by the corpus callosum (Fig. 2*b*). The corpus callosum contains both oligodendrocytes and astrocytes, so this finding excludes the possibility that Br-cadherin is highly expressed in glial cells [a probe to another brain-specific transcript was used as a control and showed that adequate amounts of RNA were present in the corpus callosum (result not shown)].

We attempted to further refine the localization of Br-cadherin by immunohistochemistry; however, the antibody cross-reacted nonspecifically on tissue sections, making interpretation of the results impossible. As an alternative approach, we cultured newborn rat glial cells and mixed glial and neuronal cells from rat hippocampi and analyzed these by immunoblotting. Results showed that Br-cadherin was absent in the primary glial cultures and was present in the mixed glial/neuron cultures (Fig. 5). This result implies expression in neuronal cells. Two antibodies to neuron-specific proteins, Ca^{2+}/calmodulin-dependent kinase II (26) and microtubule-associated protein 2 (27), clearly reacted only with the samples containing neurons, indicating that neuronal proteins were present in significant levels in the neuronal cultures and not in the glial cultures. A control antibody against a glial-specific protein, GFAP (28), reacted well with the glial extract (result not shown).

DISCUSSION

The molecular analysis of Br-cadherin defines the genomic organization and the unique spatial and temporal expression patterns of this brain-specific cadherin. Previously, we detected Br-cadherin mRNA expression in human brain (17). Here, we extend our mRNA analysis and study the protein expression and developmental timing of Br-cadherin appearance. An antibody, anti-Br-cad-EC1, was prepared against a peptide from the Br-cadherin EC1 domain. When used for immunoblotting, this antibody detected a 150-kDa protein found in human, murine, and rat brains. The predicted size of the mature Br-cadherin protein based on the cDNA sequence (starting at EC1) is 82 kDa. A discordance of calculated and observed molecular mass has been described for other cadherins (29–31), and is presumed to reflect glycosylation; Br-cadherin has four potential glycosylation sites in the predicted ORF (18). Using the polyclonal anti-Br-cad-EC1 antibody against a variety of different human and murine tissues revealed that the expression of Br-cadherin protein is restricted to the central nervous system, and no protein is detected in the human peripheral nerve. In contrast, RNA

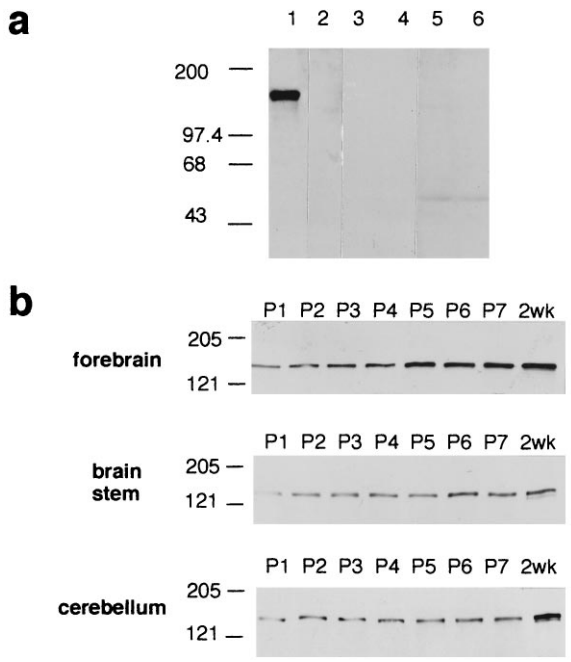

FIG. 4. Time course of expression of Br-cadherin. (*a*) Br-cadherin is not present during embryonic development and in newborn mice. Protein samples of C57BL/6J mice from adult mouse forebrain and different developmental stages were reacted with anti-Br-cad-EC1. Lanes: 1, adult forebrain; 2, 6-day-old embryos; 3, embryos from a 13-day gestation, trunk segment; 4, embryos from 13-day gestation, head segment; 5, newborns, trunk segment; 7, newborns, head segment. Six-day-old embryos were too small to dissect. (*b*) Br-cadherin initiates its expression at day 1 postnatally and gradually increases during the 1st week of life. Proteins from forebrains, brainstems, and cerebella were obtained from C57BL/6J mice at different time points postnatally and were immunoblotted with anti-Br-cad-EC1. All loadings were equalized by Comassie blue staining. Size markers are indicated in kilodaltons. P1–P7, postnatal days 1–7; 2wk, 2-week-old embryos.

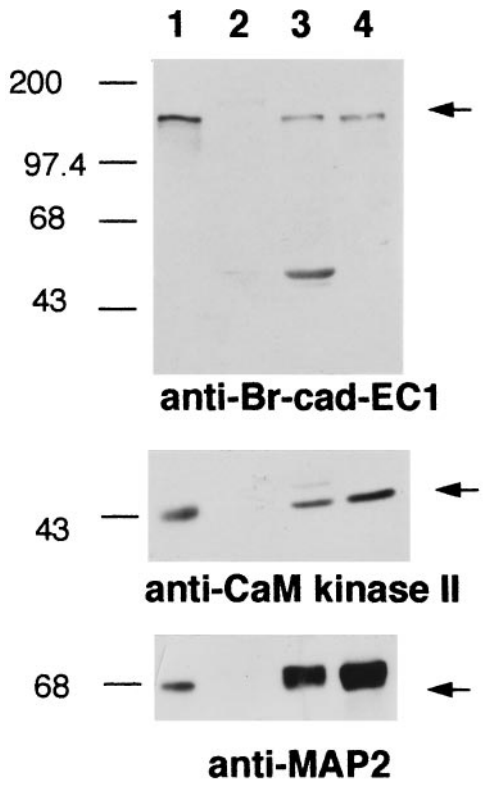

FIG. 5. Br-cadherin protein expression in neurons and glia. Extracts from cultured rat glia (lane 2) and mixed cultures of rat glia and neurons (lane 3) were reacted with anti-Br-cad-EC1. Adult rat brain (lane 1) and rat newborn hippocampus (lane 4) were reacted against the same antibody as controls. The lower molecular band detected in the glia/neurons lane is presumed to be caused by partial degradation of the sample [blotting of this sample for other high molecular proteins showed degradation products as well (results not shown)]. Identical samples to those in the *Top* were reacted with additional antibodies that detect neuron-specific proteins: anti-cell adhesion molecule kinase II (anti-CaM kinase II) (50 kDa); and anti-microtubule-associated protein 2 (anti-MAP2) (70 kDa). Size markers are given in kilodaltons.

analysis showed Br-cadherin transcripts in testis and fetal kidney as well as in the central nervous system. Br-cadherin is detected in both murine and human brains by both mRNA and protein analyses. Although at least 10 cadherins are expressed in the central nervous system, including N-, E-, R-, T-, P-, PB-cadherin, and several uncharacterized cadherins (2, 5, 13, 14), none is expressed exclusively in the brain (reviewed in ref. 6). N-cadherin, for example, is expressed in the liver, heart, muscle, kidney, and lung as well as neurons (32). Within the brain, Br-cadherin appears to be neuron-specific based on both mRNA and protein analyses; however, final confirmation of the neuronal localization will have to await development of an antibody that can be used in immunohistochemistry.

In addition to its limited range of tissue expression, another unusual feature of Br-cadherin is its pattern of temporal expression. Most other cadherins are expressed early in development, but Br-cadherin is detected relatively late. Br-cadherin is only detected postnatally in the mouse and rises to adult levels gradually over the first 2 weeks after birth. This is an extraordinarily active period in the development of the central nervous system, and the most notable change during this time is the formation of synapses (33). In the rat motor cortex, the steepest rise in synaptic density occurs between days 4 and 14 (34). In contrast to rodents, these later stages of nervous system development begin in humans during the end of the second trimester (35, 36), consistent with the low levels of Br-cadherin detected in human fetal brain. The lag in expression of Br-cadherin in the murine cerebellum in comparison to the forebrain and brainstem might reflect the relatively later development of the cerebellar circuitry (37).

In several tissues, significant levels of Br-cadherin RNA were present, but no protein was detected. The existence of protein isoforms that are missing the peptide sequence used as the antigen (the amino terminus of the first EC domain) is unlikely in the absence of any evidence for smaller, alternatively spliced mRNA isoforms, and there are no known cadherins that lack the first EC domain that contains the adhesion recognition signal (6). Thus, these results most likely indicate a posttranscriptional regulation of Br-cadherin protein levels. The unusual 5′-UTR of Br-cadherin suggests a translational control mechanism. The long Br-cadherin 5′-UTR (1089 bp) and the multiple upstream AUGs might serve to decrease translation initiation of the proper ORF (38). Alternatively, secondary structures could be formed by long 5′-UTRs, preventing the translation initiation complex from moving along the mRNA (39, 40). This mechanism of regulation has been described for several genes with long 5′-UTRs that contain multiple AUGs and are involved in developmental or tissue-specific expression (41). Several other cadherin genes possess relatively long 5′-UTRs (23, 42), so the proposed mechanism of regulation in Br-cadherin might occur in other cadherins as well.

In conclusion, our study describes the genomic organization of the only cadherin described thus far that appears to be central nervous system-specific and shows that its temporal pattern of expression would be consistent with a role during a critical period of neurogenesis. The data presented in this

study provide the tools for further exploration of the regulation of expression of this molecule and of the more general question of its role in neuronal development.

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