Fatty acid binding proteins (FABPs) constitute a multigene family of small intracellular proteins that bind hydrophobic ligands. In this report we describe the cloning and expression pattern of a novel member of this gene family that is specifically expressed in the developing and adult nervous system and thus was designated brain (B)-FABP. B-FABP is closely related to heart (H)-FABP with 67% amino acid identity. B-FABP expression was first detected at mouse embryonic day 10 in neuroepithelial cells and its pattern correlates with early neuronal differentiation. Upon further development, B-FABP was confined to radial glial cells and immature astrocytes. B-FABP mRNA and protein were found in glial cells of the peripheral nervous system such as satellite cells of spinal and cranial ganglia and ensheathing cells of the olfactory nerve layer from as early as embryonic day 11 until adulthood. In the adult mouse brain, B-FABP was found in the glia limitans, in radial glial cells of the hippocampal dentate gyrus and Bergman glial cells. These findings suggest a function of B-FABP during neurogenesis or neuronal migration in the developing nervous system. The partially overlapping expression pattern with that of cellular retinoid binding proteins suggests that B-FABP is involved in the metabolism of a so far unknown hydrophobic ligand with potential morphogenic activity during CNS development.

Key words: fatty acid binding protein, gene isolation, radial glia, ensheathing cells, satellite cells, neuroepithelium, CNS development, mouse

INTRODUCTION

Fatty acid binding proteins (FABPs) constitute a multigene family of small intracellular proteins that bind hydrophobic ligands such as fatty acids, eicosanoids and retinoids. More than 12 different FABPs have been described (Börchers and Spener, 1994) and by sequence homology they can be classified into 4 major subfamilies: heart-, liver- and intestinal type FABPs as well as the group of cellular retinoid binding proteins (CRtBPs).

FABPs, in general, are synthesized in specific differentiated tissues (Matarese et al., 1989; Veerkamp et al., 1991). The physiological roles of FABPs remain imperfectly understood while structural diversity, tissue and ligand specificity suggest distinct functional specializations (Kaikas et al., 1990). Evidence supports a dual function: alleviation of intracellular transport and metabolism of their hydrophobic ligands and sequestration of ligands in a manner that limits their association with alternative binding sites, in particular with members of the nuclear hormone receptor superfamily such as the retinoic acid receptors (RAR, RXR), and the peroxisome proliferator activated receptors (PPAR). In the case of CRtBPs, evidence has been provided that the binding protein-ligand complexes act as direct substrates in several steps of retinoid conversion (Napoli, 1993; Posch et al., 1992). Thereby, these proteins may be implicated in the generation of retinoic acid gradients. It was suggested that other FABPs might fulfill a similar role with respect to hydrophobic ligands, such as fatty acids or their metabolites, for other members of the large nuclear hormone receptor family such as PPAR or one of the ‘orphan’ receptors (Bass, 1993; Issemann et al., 1992). This hypothesis is based on observations that expression of liver- and adipocyte-FABP is induced by fatty acids in hepatocytes and adipocytes (Kaikas et al., 1993; Grimaldi et al., 1992), respectively, that PPAR induces FABP expression in hepatocytes (Brandes et al., 1990), that overexpression of liver-FABP in hepatoma cells increased their growth response to linoleic acid (Keler and Sorof, 1993), and that fatty acids and eicosanoids may activate PPAR and RXR (Eager et al., 1992; Keller et al., 1993).

We have been studying the function of mammary derived growth inhibitor (MDGI) (Böhmer et al., 1987), which is
identical to heart (H)-FABP (Börchers and Spener, 1994). This protein is constitutively expressed in heart and skeletal muscle of postnatal animals, but becomes induced in an endocrine hormone-dependent manner in differentiating mammary epithelial cells during pregnancy and lactation (Kurtz et al., 1990). H-FABP inhibits the proliferation of mammary epithelial cells in vitro, and stimulates functional differentiation and branching morphogenesis of mammary ducts in mammary gland organ culture (Grosse et al., 1992). Furthermore, pre-treatment of embryonic stem cells with H-FABP resulted, after transplantation into syngeneic mice, in teratocarcinomas, which contained decreased proportions of undifferentiated embryonic carcinoma cells and significantly more differentiated neuronal tissue (Wobus et al., 1990). It was conceivable that H-FABP mimicked the action of a related protein, which might be functional during development of the nervous system. This assumption was supported by evidence of the existence of a H-FABP related protein in brain (Bass et al., 1984; Schoentgen et al., 1990; Heukeroth et al., 1987).

Here, we describe the identification and isolation of a new mouse gene encoding brain-FABP (B-FABP) and its expression pattern in embryos and adult mice. Spatiotemporal gradients of B-FABP gene expression were observed in the developing nervous system that correlate with neuronal and glial cell differentiation. Our findings suggest a function of B-FABP during the development of the nervous system.

**MATERIAL AND METHODS**

**Animals**

Adult and pregnant NMRI mice of defined gestational stage were obtained from Charles River. Mice were mated overnight and conception was confirmed by the presence of a vaginal plug. The morning of the day after conception was regarded as embryonic day E0, the day of birth as P0.

**Isolation and characterization of the B-FABP gene**

The degenerate oligonucleotides Gs and Ga were synthesized (cf. Fig. 2) corresponding to consensus peptide sequences STFKNTEI and VQKW(N/D)(K/Q) which are conserved in H-FABP, adipocyte (A)-FABP and myelin P2 (Hunt et al., 1986; Narayanam et al., 1991). 100 ng of Balfic mouse genomic DNA was amplified by polymerase chain reaction (PCR) for 30 cycles with these primers. The three main products were cloned and sequenced. Fragment 2 resulted from a novel FABP gene. This fragment was used to screen a λGEM11 mouse genomic library (Promega). Three phage clones were isolated that all had identical restriction patterns. Overlapping HindIII, SacI and EcoRI fragments from these clones were subcloned into pGEM3Z and phBluescript SK(+) and sequenced in both directions using Sequenase 2.0 (United States Biochemicals) and T-deaza-dGTP. The identified gene encoding brain (B)-FABP was designated Fabpb (GenBank U04827).

The B-FABP cDNA coding sequence was amplified by PCR using the oligonucleotides Rs and Ra, derived from the genomic sequence (Fig. 2), and 200 ng of reverse transcribed total brain RNA. This fragment was ligated into the HindII site of pGEMZ and sequenced. The transcription start site was determined by primer extension as described by Sambrook et al. (1989). The 3' end of the mRNA was determined by 3'-RACE according to Frohman et al. (1988) with the nested primers Cs1 and Cs2 from the 4th exon of Fabpb.

Sequences of oligonucleotides used in this study are: Gs (5'-ACITTYAARAYACIGGRAT), Ga (5'-YTKICCRTYCCAYT-TYTGIAAC), Rs (5'-ATGGTAGATGCCTTTGC), Ra (5'-CATGCG-CTTTCTAATACGAGGCA), Pa (5'-TGCAGTCTGCTGCAGT- TCCAGGTGTGGCGCA), Cs1 (5'-ATCCAGATTGTTGGAATAA), Cs2 (5'-CACGCTGAAATGTCATTAGG).

**Northern analysis and in situ hybridization**

10 μg of total RNA from different mouse tissues was isolated (Chomczynski and Sacchi, 1987), separated on a 1% agarose/formaldehyde gel, and transferred onto nitrocellulose membranes (Hybond-C, Amersham). Equal loading of lanes was confirmed by densitometric comparison of the ethidium bromide stained 28S rRNA band. Baked membranes were hybridized with a 35S labelled B-FABP probes in 6x SSC, 5x Denhardt solution, 0.5% SDS, 50 μg/ml denatured salmon sperm DNA overnight at 60°C. Membranes were washed in 0.1x SSC, 0.1% SDS three times for 15 minutes at 60°C and analysed using an Fujix Bio-imaging analyser BAS 2000. In situ hybridization to cryostat sections was performed as described previously (Kurtz et al., 1990). 8-10 μm sections were postfixed in 4% paraformaldehyde for 1 minute and hybridized in 2x SSC, 50% formamide, 10% dextran, 10 mM DTT, 1 μg/ml tRNA, 1 μg/ml sheared herring sperm DNA, 2 μg/ml BSA containing 1x105 cpm 35S-labelled RNA probes (specific activity >109 cpm/μg) at 55°C for 3 hours. Slides were washed at 52°C in 2x SSC, 50% formamide, rinsed in 2x SSC and incubated in RNase A (100 μg/ml) for 30 minutes at 37°C. After final washes, sections were dehydrated and exposed to Kodak NB3 film after autoradiography for 3 days. Sections were stained with toluidine blue. Microphotographs were taken in bright- and dark-field illumination (Zeiss Axiophot).

**Immunohistochemistry**

The generation of anti B-FABP antibodies will be described elsewhere (F. Schmutzgen, manuscript in preparation). Briefly, recombinant B-FABP was produced in bacteria using the pET-vector system (Studier et al., 1990). Rabbits were injected twice with the purified recombinant B-FABP and recombinant protein. B-FABP-specific antibodies were purified from the antiserum by affinity chromatography using a resin coupled with recombinant B-FABP. Specificity of the antibodies was confirmed by western blot analysis.

Adult mice were deeply anesthetized with ether and perfused transcardially with fixative, which for cryosections consisted of 2% paraformaldehyde, 75 mM lysine, 10 mM periodate in 0.1 M phosphate buffer pH 7.4, and for paraffin embedding, 10% formaldehyde were used. Tissues were dissected, postfixed overnight at 4°C, and processed as described previously. Infants were exposed to Kodak NB3 film after autoradiography for 3 days. Sections were stained with toluidine blue. Microphotographs were taken in bright- and dark-field illumination (Zeiss Axiophot).
with species-specific secondary antibodies conjugated either to Cy3 (rabbit) or DTAF/HTC (mouse) in 2% normal goat serum, 0.1% Triton X-100 in PBS. For double labelling with Rat-401, samples were incubated sequentially with anti-B-FABP Ig, anti-rabbit IgG × Cy3, Rat-401 and anti-mouse IgG × DTAF. Photomicrographs were taken on a Zeiss axiophot microscope. Original slides were scanned on Kodak Photo CD, processed with Adobe Photoshop, and printed on a Tektronics PhaserlISDX Dye Sublimation Printer.

RESULTS

Identification and characterization of the mouse B-FABP gene

Based on their sequence similarity, H-FABP, adipocyte (A−) FABP, and myelin P2 can be grouped in one distinct FABP subfamily. This subfamily contains two stretches of conserved amino acids, which are encoded by exons two and three. These stretches are separated by unique sequences within the first and third exons. Three main PCR products obtained (Fig. 1) were identified by their intron size and sequence as fragments from the H-FABP gene and pseudogene (fragments 3 and 1) (Treuner et al., 1994) and as a novel FABP gene sequence (fragment 2). Fragments 1 and 3 resulted from the H-FABP gene and pseudogene, respectively. Fragment 2 arose from the newly discovered gene Fabpb. Markers (to the left of lane 1) correspond, from bottom to top, to bands of 125, 564, 831, 947, 1,375 and 1,584 bp.

Fig. 1. Identification of the novel gene Fabpb. The degenerate oligonucleotides Gs and Ga were used to amplify intron fragments of FABP genes from mouse genomic DNA by PCR. Three main PCR products were separated by agarose electrophoresis and visualized by ethidium bromide staining (lane 2). Fragments 1 and 3 resulted from the H-FABP gene and pseudogene, respectively. Fragment 2 arose from the newly discovered gene Fabpb. Markers (to the left of lane 1) correspond, from bottom to top, to bands of 125, 564, 831, 947, 1,375 and 1,584 bp.

B-FABP gene expression in adult mice

Using the complete B-FABP cDNA fragment as a probe, northern analysis of adult mouse tissues was performed (Fig. 4). B-FABP mRNA was only detected in brain, particularly in the olfactory bulb. No B-FABP mRNA could be identified in the sciatic nerve. In contrast, H-FABP expression was detected in a variety of tissues, including the brain, but predominantly in heart and lactating mammary gland.

The regional and cellular localizations of B-FABP mRNA and protein were identified by the combination of in situ hybridization and immunohistochemistry. In the PNS, high levels of B-FABP mRNA were identified in cranial and spinal ganglia of adult mice (Fig. 5A,B). Immunohistochemistry revealed that satellite cells express B-FABP, while neurons and Schwann cells were negative (Fig. 6A). Particularly high levels of B-FABP mRNA and protein were detected in the olfactory nerve from just beneath the olfactory epithelium up to the glomerular layer of the olfactory bulb (Figs 5C,D, 6B). Immunostained cells appeared fibrous. No labelling was observed in the olfactory epithelium. Thus, B-FABP is expressed most likely in ensheathing cells (Doucette, 1989; Pixley, 1992).

In the CNS, B-FABP mRNA hybridization was detected above the Purkinje cell layer and to a lesser extent over the molecular layer of cerebellum (Fig. 5E,F). B-FABP immunohistochemistry identified Bergman glial cells (Fig. 6C). In the hippocampus, radial glial cells, which span the granule cell layer, were found to be B-FABP positive (Fig. 6D). Furthermore, B-FABP was detected in the glia limitans (Fig. 5C) and
some astrocytes throughout the CNS showed low B-FABP immunoreactivity in their cell bodies (Fig. 6B).

The specificity of in situ hybridization signals was confirmed by control hybridization with a B-FABP sense-riboprobe and a H-FABP antisense probe. In the former case no signal over background could be detected. When the probe for H-FABP was used, a strikingly different hybridization pattern was observed (not shown). Specificity of B-FABP immunohistochemistry was confirmed by competition with recombinant B-FABP and comparison with the staining pattern obtained with anti-H-FABP antibodies. The latter stained muscle tissue and in the CNS choroid plexus. These structures were negative with the anti-B-FABP antibodies (not shown).

B-FABP gene expression in the developing nervous system

B-FABP gene expression was found as early as day 10 of mouse embryogenesis in the spinal cord, hindbrain, midbrain, dorsal root ganglia (Fig. 7A,B), and the trigeminal ganglion (not shown). In the forebrain region, only the ganglionic eminence was markedly labelled (Fig. 7A,B). Only low levels of B-FABP protein were detectable at this stage (not shown). Starting from this stage, we studied the temporally changing pattern of B-FABP gene expression in selected regions of the embryonic nervous system.

Spinal cord

At embryonic day E10, B-FABP mRNA (not shown) and low amounts of protein were distributed throughout the width of the neuroepithelial layer in the dorsal spinal cord (Fig. 8A). Thin stripes of strongly immunoreactive cells were detected in the most ventral ventricular zone in immediate neighborhood to the floor plate. Immunoreactive radial processes framed the

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**Fig. 2.** Sequence of Fabpb. Exon regions are printed in capital letters while portions of the introns, 5′- and 3′-flanking regions are shown in lower case letters. The deduced amino acid is placed under the middle nucleotide of each codon. The location of oligonucleotides Gs and Ga, used for amplification of the intron fragment (underlined), and the oligonucleotides Rs and Ra, used for amplification of the cDNA fragment, are indicated by horizontal arrows below the genomic sequence. The putative TATA-box and polyadenylation signal are double-underlined. (GenBank accession number: U04827).

**Fig. 3.** Specificity of the anti-B-FABP antibodies: 200 ng of isolated rat H-FABP (PH), 200 ng of purified recombinant mouse B-FABP (PB), and 10 µg protein each of cytoplasmic extracts from lactating mammary gland (CH), as a source of H-FABP, and from olfactory bulbs (CB), as a source of native B-FABP, were separated by SDS-electrophoresis and transferred onto nitrocellulose. Parallel blots were immunostained with affinity purified anti-rat H-FABP antibodies and anti-B-FABP antiserum. The electrophoretic mobility of B-FABP (apparent molecular mass 15.5 × 10^−3) is slightly lower than that of H-FABP (14.5 × 10^−3). Both antibodies preferentially recognize their cognate antigens. No cross-reactivity with other components of the cytoplasmic extracts was detected.
floor plate. No hybridization signal or immunoreactivity was seen in the floor plate.

The B-FABP-positive cells framing the floor plate became more prominent at E11 (Fig. 8B). Commissural neurons were detected by their NF160 immunoreactivity at this stage. Between E12 and E14, the ventricular zone narrows in a ventrodorsal temporal gradient (Nornes and Das, 1974). In parallel, B-FABP producing cells with immunoreactive radial processes became restricted to the narrowed ventricular zone. At day E12, regression had reached the ventral half of the thoracic spinal cord (not shown) and at E14 the entire ventricular zone became limited to a thin layer of strongly B-FABP-

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**Fig. 4.** Tissue specificity of Fabpb expression in adult mice. RNA samples from various mouse tissues were analysed by northern blots. Parallel blots were hybridized with B-FABP and H-FABP cDNA probes. After hybridization and washing, they were exposed to imaging screens for 3 hours (H-FABP) and 15 hours (B-FABP). Hybridizing bands of about 900 nt (B-FABP) and 850 nt (H-FABP) are shown. k, kidney; ag, adrenal gland; t, testis; sp, spleen; sm, skeletal muscle; li, liver; lu, lung; h, heart; mgl and mgp, mammary gland from lactating and pregnant mice, respectively; b, whole brain; n, sciatic nerve; ob, olfactory bulb.

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**Fig. 5.** In situ hybridization analysis of B-FABP gene expression in the nervous system of adult mice. Photomicrographs were taken from each sample under dark-field (A,C,E) and bright-field illumination (B,D,F). (A,B) Trigeminal ganglion: a patchy hybridization signal can be associated with satellite cells. A region of mesodermal tissue (*) in the section is not labelled. (C,D) Sagittal section through the olfactory bulb: extensive hybridization revealed in the olfactory nerve (on), which extends from the olfactory epithelium (*) to the glomerular layer (arrowheads) of the olfactory bulb. Low hybridization signal is detected over the pia mater (arrows). (E,F) Cerebellum: the hybridization signal is mainly detected above the Purkinje cell layer (arrowheads) but weak hybridization is also visible above the molecular layer (ml). No B-FABP mRNA is found in the granule cell layer (gcl). Bar: 100 µm (A,B,E,F), 200 µm (C,D).
positive cells, which extended immunoreactive radial processes to the pial surface (Fig. 8E).

B-FABP-positive radial cells also immunoreacted with the radial glial cell-specific antibody RC2 (not shown) and with the antibody Rat-401 (Fig. 9D) against nestin, which is expressed in neural precursor cells and radial glial cells. But in contrast to the B-FABP distribution, RC2 and Rat-401 also labelled radial processes in the floor and roof plate.

At day E17, the B-FABP immunoreactivity was no longer apparent in the ventricular zone and many B-FABP-positive cells with multiple processes or monopolar cells attached to the pial surface could be seen (not shown). These phenotypes resembled those described for the transition from radial glial cells to astrocytes (Cameron and Rakic, 1991). Postnatally, B-FABP synthesis was reduced but still detectable in GFAP-positive astrocytes in the spinal cord (not shown).

In situ hybridization demonstrated that the pattern of B-FABP mRNA was congruent with the protein distribution (Fig. 9A). Silver grains were distributed with a low density over the whole dorsal ventricular zone, but were highly concentrated around the floor plate. In particular, those radial processes lining the floor plate contained high levels of B-FABP mRNA and formed a clamp-like structure. This clamp-like organization extended rostrally up to the met-/mesencephalic boundary. Double-labelling with anti-NF160 antibodies demonstrated the cytoarchitecture in the floor plate region (Fig. 9B). The corticospinal tract with its descending axon bundles was apparently separated from the floor plate by an interspace, which is formed by strongly B-FABP-positive radial processes. These originated in the ventricular zone just besides the floor plate. Decussating axons crossed this interspace and passed the midline below the floor plate.

Mid- and forebrain

In the mesencephalon at embryonic day E11, B-FABP synthesis was found throughout the width of the neuroepithelium in the lateral walls but not in the ventral and dorsal midline regions (Fig. 10A). A thin subpial layer of early neuronal cells, identified by MAP2 immunoreactivity (Fig. 10B), was found only above the B-FABP-positive ventricular zone regions. During further develop-

![Fig. 6. Identification of B-FABP synthesizing cell types in the nervous system of adult mice. A paraffin section (A) and cryostat sections (B-D) were stained by double immunofluorescence (B, D) and immunoperoxidase/hematoxylin (A, C). (A) Trigeminal ganglion and nerve: B-FABP specific immunostaining identifies satellite cells (arrowheads) surrounding the cell bodies of ganglion neurons (*). Schwann cells are not labelled. (B) Sagittal section through the olfactory bulb and the attached olfactory nerve: extensive B-FABP immunofluorescence labelling (red) is visible throughout the olfactory nerve (on). In the glomerular layer (gl) and the granule cell layer (gcl) of the olfactory bulb, astrocytes contain GFAP immunoreactivity (green) in their processes and low amounts of B-FABP in their cell bodies (arrows). In the external plexiform layer (epl), GFAP staining of astrocytes is lower and B-FABP immunoreactivity is more prominent (double arrows). (C) Cerebellum: immunoperoxidase staining of Bergman glial cells with anti-B-FABP antibodies shows their strongly labelled cell bodies surrounding the Purkinje cells (arrows). Slightly immunoreactive processes originating from Bergman glial cells project through the molecular layer (ml). No staining is apparent in the granule cell layer (gcl). Photomicrograph was taken with Nomarski optics. (D) Dentate gyrus of hippocampus: radial glia cells of the dentate gyrus are double labelled with anti-B-FABP (red) and anti-GFAP antibodies (green). B-FABP is mainly detected in cell bodies that line the hilar surface (h, hilus) of the granule cell layer (gcl).]
2643 B-FABP expression during CNS development

pocampal formation and in the ventricular zone of the olfactory bulb (Fig. 7C-F). Only minor amounts of B-FABP were detected in the dorsal telencephalon until day E15. B-FABP was never found in the ventral and dorsal midline regions of the diencephalon (Fig. 7C, D). In contrast, nestin was expressed in radial cells throughout the CNS at early developmental stages (Hockfield and McKay, 1985; Frederiksen and McKay, 1988).

Double labelling immunofluorescence experiments were performed with anti-MAP2 antibodies to identify early neuronal differentiation. At embryonic day E11 and E12, accumulation of MAP2-positive cells was first detected in the intermediate zones of the hypothalamus and of the ventrolateral domain of the lateral ventricle (Fig. 11B, C). In both areas, high levels of B-FABP were synthesized. In the hypothalamus and in the striatum several B-FABP-positive cells could be observed between embryonic day E12 and E14 which had apparently moved into deeper layers of the ventricular wall. These cells did not stain for either the neuronal marker MAP2 or for the precursor cell marker nestin (Fig. 11D, E).

At day E17, the thin-layered ventricular zone throughout the di- and telencephalon was evenly B-FABP positive and typical radial glial cells could be observed. However, with the exception of a thin layer of cells along the dentate gyrus, no radial glial cells were stained in the hippocampus (not shown). Between the day of birth (P0) and P10 the transition from B-FABP-positive, GFAP-negative radial glial cells to GFAP-positive astrocytes could be observed in the cortex (Fig. 11F, G).

Cerebellum

Immunohistochemistry identified monopolar cells with a loose laminar organization at postnatal day P0 (Fig. 11H) and at P10, B-FABP-positive cells enclosed the Purkinje cells and extended processes through the molecular layer and the external germinal layer to the pial surface. These processes were double labelled with GFAP (Fig. 11I). These features identify Bergman glial cells. In the molecular layer a meshwork of B-FABP immunoreactivity was seen.

Peripheral nervous system

From the earliest time point studied, E10, cranial and dorsal root ganglia were found to contain B-FABP mRNA (Fig. 7A-D). Labelling was preserved throughout all developmental stages until adulthood. At embryonic day E11, double labelling immunofluorescence discriminated NF160-positive neuronal cells from B-FABP-positive glial cells in the ganglia (Fig. 8B, C). Throughout development highest B-FABP levels were detected in the trigeminal ganglion.
B-FABP immunoreactivity and mRNA could be detected in the olfactory nerve as early as day E11 (Figs 7E,F, 11A). B-FABP was always excluded from the olfactory epithelium.

DISCUSSION

B-FABP is structurally related to the heart-type FABP family

In this study we have cloned and characterized the gene *Fabpb* encoding a novel member of the fatty acid binding protein family, brain (B)-FABP, that is specifically expressed in the nervous system. This project was initiated by the finding that H-FABP promotes the differentiation of embryonic stem cells into neuronal cells (Wobus et al., 1990), suggesting that H-FABP or a closely related protein plays an important role in neuronal differentiation. The existence of a brain-specific FABP was indicated by previous data (Bass et al., 1984; Heuckeroth et al., 1987; Schoentgen et al., 1990).

To isolate *Fabpb*, we took advantage of the fact that the gene structure of FABPs is highly conserved (Matarese et al., 1989). Degenerate PCR primers were used to amplify the second introns of FABP genes, which could be discriminated by their size. One PCR

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**Fig. 8.** Temporal changes of the B-FABP distribution in spinal cord. Photomicrographs show transverse sections through spinal cord at the upper thoracic-cervical level. Cryostat sections were labelled by immunofluorescence (B-FABP, red). (A) E10 embryo: B-FABP is detected throughout the neuroepithelial layer in the dorsal region (top) and in radially appearing cells (arrowheads) juxtaposed to the floor plate (*). (B) E11 embryo: B-FABP and NF160 (green) double immunofluorescence; B-FABP expression is more prominent in a clamp-like formation around the floor plate (*) which is formed by ventral neuroepithelial cells and their immunoreactive processes (arrowheads). Commisural axons (c) are immunostained with anti-NF160 antibodies (green). bp, basal plate; drg, dorsal root ganglion. Boxed area is shown in higher magnification in C: B-FABP-positive cells are most likely satellite cells and are clearly distinguished from NF160-positive neuronal cells. (D) Floor plate area at E12, double immunofluorescence with anti-B-FABP and Rat-401 (green), scanned with a confocal microscope: Radial cells (arrowhead) framing the floor plate (*) are double labelled as indicated by their yellow appearance. Radial processes within the floor plate react only with Rat-401. c, central canal of spinal cord. (E) Transverse section through spinal cord at E14: The ventricular zone (arrowheads) became limited to a thin layer of B-FABP-expressing cells, which extend B-FABP immunoreactive radial processes to the pial surface (p). The floor plate (*) is immunonegative. Bar: 100 µm (A,B,E), 50 µm (C,D).

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**Fig. 9.** Cytoarchitecture of the clamp-like structure formed by B-FABP expressing radial cells around the floor plate. (A) In situ hybridization of transverse section through the spinal cord at embryonic day E11: B-FABP mRNA is strongly expressed in the ventral part of the ventricular zone and in radial processes (arrowheads) lining the floor plate (*). A low density hybridization signal is seen above the more dorsal ventricular zone (vz). drg, dorsal root ganglion. (B) Double immunofluorescence for B-FABP (red) and NF160 (green) of a transverse section through the midline region of the medulla oblongata: Axon bundles of the pyramidal tracts (p) are separated from the floor plate (*) by an interstice, which is formed by strongly B-FABP-positive radial processes (arrowheads). These processes originate in the ventricular zone (vz) close to the floor plate. Decussating axons (arrows) cross this interstice and pass below the floor plate. IV, fourth ventricle. Bar: 100 µm.
fragment was found to result from a novel FABP gene and was used to isolate *Fabpb* genomic clones.

B-FABP is most closely related to H-FABP. Those amino acid residues that were identified by crystallographic studies as significant for ligand binding of heart-type FABP are conserved. The structural similarity of B-FABP and H-FABP suggests that both proteins bind similar ligands. It was shown that fatty acids bound to human H-FABP adopt a bend U-shape (Zanotti et al., 1992). Binding of unsaturated fatty acids such as arachidonic acid seems to be preferred. Binding of retinoic acid was described for A-FABP and myelin P2 (Uyemura et al., 1984; Matarese and Bernlohr, 1988).

Recently, a cDNA was isolated encoding a FABP in the retina of chicken (Godbout, 1993). It is equally homologous to mouse H-FABP (Treuner et al., 1994) and B-FABP. We could not detect B-FABP in the developing mouse retina. Screening for developmentally regulated genes in the mouse cerebellum yielded the H-FABP related cDNA clone, GC9, with an expression pattern similar to B-FABP (Kuhar et al., 1993).

**Spatiotemporal pattern of B-FABP expression correlates with differentiation of neuronal and glial cells**

We have shown by in situ hybridization and immunohistochemistry that B-FABP mRNA and protein exhibit a distinct, temporally and spatially restricted expression pattern in the embryonic and adult nervous system. Our findings establish a correlation between B-FABP expression in neuroepithelial precursor cells and early neuronal differentiation.

At early developmental stages, high levels of B-FABP expression in the forebrain were identified in a ventrolateral domain of the lateral ventricle. In the same area neuronal cells accumulate early (Ferri and Levitt, 1993). Evidence has been provided that retinoids may be implicated in the early induction of neurogenesis in the forebrain (Zimmer and Zimmer, 1992). In transgenic mice, the β-galactosidase gene under control of a retinoic acid response element (RARE) was found to be expressed in neuroepithelial cells of this ventrolateral domain and in the developing olfactory pit (LaMantia et al., 1993). In both areas, RARE activity and neuronal induction were correlated. In both regions B-FABP as well as CRtBPs are enriched (Gustafson et al., 1993).

In the striatum and in the hypothalamic area, clusters of B-FABP-positive cells could be seen in the intermediate zone between days E12 and E14. They were either arranged radially or dispersed. In the latter case, cells had a monopolar phenotype and the trailing process was oriented perpendicular to the orientation of the radial glial processes. Even though these cells did not express MAP2, the time of their occurrence and their phenotype would suggest that they are early neuronal cells.

B-FABP mRNA and protein levels are present throughout the width of the pseudostratified columnar neuroepithelium of spinal cord, hind- and midbrain at embryonic day E10. However, no expression is detected in the ventral and dorsal midline regions. Early neuronal cells, which were identified by their MAP2 immunoreactivity, begin to differentiate in a thin subpial layer of the mesencephalic ventricular wall just above the region where B-FABP is found in the ventricular zone. Further neuronal differentiation starts at the ventral boundary of the B-FABP expressing region and propagates dorsally. In parallel with the ventral to dorsal propagation of neuronal differentiation, B-FABP becomes enriched in radial glial cells which span the MAP2-positive ventricular wall.

In the hindbrain and spinal cord, high concentrations of B-FABP accumulate during early developmental stages in a thinned layered clamp-like organization which encloses the floor plate. This structure is formed by radial cells in the most ventral ventricular zone juxtaposed to the floor plate. The floor plate has been implicated in the homing of commissural axons, probably by producing a diffusible chemoattractant (Placzek et al., 1990). Furthermore, the floor plate may cause a change in trajectory of commissural axons (Bovolenta and Dodd, 1990). The increase of the B-FABP level in cells of the ventricular...
zone and their radial processes propagates dorsally together with the wave of neuronal differentiation and regression of the ventricular zone. A similar spatiotemporal expression pattern was observed for F-spondin, which is a secreted glycoprotein promoting cell adhesion and neurite outgrowth (Klar et al., 1992). The observed correlation of B-FABP and F-spondin expression and early neuronal differentiation and migration suggests a function of B-FABP in these processes.

The radial appearance of B-FABP-positive cells resembles the phenotype of radial glial cells (Edwards et al., 1990). Indeed, they are double labelled with the radial glial-specific antibody RC2 (Misson et al., 1988) and with the anti-nestin antibody Rat-401 (Hockfield and McKay, 1985), which labels neuronal precursor cells and radial glia. These antibodies preferentially stain cellular processes. As a cytoplasmic protein, B-FABP is mainly localized within the cell bodies and radial

![Fig. 11. B-FABP synthesis in the developing olfactory nerve and brain. (A) Transverse section through the olfactory bulb (ob) and olfactory epithelium (oe) at embryonic day E12: the olfactory nerve (arrowhead) and the ventricular zone of the olfactory bulb express B-FABP. No labelling is detected within the olfactory epithelium. (B) Double immunofluorescence labelling of B-FABP (red) and MAP2 (green) in a transverse section through the ganglionic eminence (striatum) at day E11. B-FABP expression is evident in the ventricular zone (vz) of the striatum and ceases more dorsally. Neuronal differentiation, as indicated by the MAP2 immunoreaction, is most advanced in the striatum (arrowheads). lv, lateral ventricle. (C) Transverse section of the hypothalamus at embryonic day E12, double immunofluorescence for B-FABP (red) and MAP2 (green): accumulation of MAP2 immunoreactive neuronal cells appears first in areas where the ventricular zone cells contain high levels of B-FABP. III, third ventricle. (D) Hypothalamus, E12 embryo: single B-FABP-positive, MAP2-negative cells had apparently separated from the ventricular zone and migrated into deeper layers of the ventricular wall (arrowhead). (E) Transverse section of the striatum at day E12. Radial glial cells are double labelled with anti-B-FABP (red) and anti-nestin Rat-401 (green). B-FABP labelling dominates in their cell bodies located in the ventricular zone (*), Rat-401 labelling is dominant in their radial processes. Individual B-FABP-positive, nestin-negative cells are apparent in the striatum (arrowhead). Some of these cells carry processes that are oriented perpendicular to the radial fibers. (F) Sagittal section of the cerebral cortex at P0, B-FABP immunofluorescence: B-FABP is expressed in radial glial cells. Early astrocyte precursors are scattered throughout the cortical wall. lv, lateral ventricle; vz, ventricular zone; cl, cortical layer. (G) Sagittal section of the cerebral cortex at day P10, double immunofluorescently stained for B-FABP (red) and GFAP (green): GFAP-positive astrocytes contain residual levels of B-FABP mainly in their cell bodies (arrowhead). (H) Sagittal section of postnatal day P0 cerebellum: immunofluorescence for B-FABP (red) and Hoechst33258 staining of cell nuclei (blue): B-FABP-positive immature Bergman glial cells extend processes through the molecular layer (ml) and the external germinal layer. (I) Sagittal section of the cerebellum at P10, double immunofluorescence for B-FABP (red) and GFAP (green); B-FABP expression is prominent in Bergman glial cells which are juxtaposed to Purkinje cells (arrowheads). Bergman glial cells extend double labelled processes through the molecular layer (ml) and the external germinal layer (egl) to the pial surface. Double labelling is most prominent in the pial endfeet (arrows). Some B-FABP background labelling of unresolved cellular origin can be seen in the granule cell layer. Bars: 100 µm (A-C,F), 50 µm (D,E,G-I).
processes are only faintly immunoreactive. To assure co-localization of B-FABP and nestin we have used confocal laser scanning microscopy. In contrast to the B-FABP distribution, RC2 and Rat-401 label radial cells throughout the ventrodorsal axis. In particular, these antibodies also stain radial glial cells in the ventral and dorsal midline of the neural tube where no B-FABP is detectable. Apparently, B-FABP is expressed during embryonic CNS development by a subpopulation of RC2 and nestin-positive radial precursor cells in those regions of the neural tube where neuronal cells originate.

The B-FABP staining pattern in the ventral half of the spinal cord at embryonic day E12 and later stages resembles the pattern observed with the antibody A2B5 (Frederiksen and McKay, 1988), which labels glial precursor cells. Evidence has been provided that oligodendrocyte and astrocyte precursors are preferentially localized in the ventral spinal cord during early stages of development (Fok-Seang and Miller, 1994). We observed transitional forms between B-FABP-expressing radial glial cells and astrocytes in the spinal cord and hindbrain at E17 and postnatally in the cerebral cortex in concordance with previous observations (Cameron and Rakic, 1991). We assume that B-FABP expression is confined to radial glial cells at later stages of development. The question of whether early neuronal precursor cells also express B-FABP will be addressed in future experiments. Early glial cells could be distinguished from neuronal cells in dorsal root and cranial ganglia at E11 by complementary staining of cells with either B-FABP-specific or neurofilament-specific antibodies. The rootlets of sensory nerves were also immunoreactive at early stages of development (not shown). This might indicate that B-FABP transiently labels common precursor cells of satellite and Schwann cells (Frank and Sanes, 1991). Mature Schwann cells do not contain B-FABP. Instead, they express the related myelin P2 (Narayanan et al., 1991). This represents an interesting correlation of differential gene expression and differentiation. Throughout development until adulthood, B-FABP levels remain high in these peripheral glial cell lineages.

The idea that B-FABP might facilitate neuronal differentiation, migration or axon extension is further supported by its expression pattern in the adult nervous system. The olfactory receptor neurons are replaced continuously in the adult animal. Their axons grow to the glomerular layer of the olfactory bulb. They are enclosed by the strongly B-FABP expressing ensheathing cells. In the adult hippocampal formation, neurogenesis and expression of B-FABP are limited to the dentate gyrus. In the cerebellum, B-FABP is present in the Bergman glial cells and their processes, a particular class of radial glial cells, which serve to guide granule cells and which persist into adulthood. Recently, it was shown that in the adult cerebellum these cells preserve the ability to guide migrating neurons (Sotelo et al., 1994).

**Potential function of B-FABP**

With respect to function, the FABP subfamily comprising two retinol (CRBP I and II) and two retinoic acid binding proteins (CRABP I and II) is best characterized. Evidence has been provided that the binding protein-ligand complexes act as direct substrates in several steps of retinoid conversion (Napoli, 1993; Posch et al., 1992). In this way, these proteins apparently regulate the availability of all-trans-retinoic acid, which serves as a ligand for the retinoic acid receptor RAR, and after isomerisation into 9-cis-retinoic acid, as a ligand for RXRs. Retinoic acid is known as a potent morphogen in the developing nervous system (Morriss-Kay, 1993). The expression pattern of B-FABP in the developing nervous system partially overlaps with that of CRBP and behaves in a complementary manner to that of CRABP in several respects (Maden et al., 1990; Ruberte et al., 1993). Similar to B-FABP, CRBP I expression in the spinal cord is first detected in the ventral ventricular zone and extends dorsally. CRABP I is found early in commissural neurons. Both CRBP I and CRABP I are found early in spinal as well as cranial ganglia. In the forebrain at E12, CRBP I and B-FABP are concentrated in the neuroepithelial layer of the striatum, while CRABP I and CRBP I are found in the corpus striatum. Both CRBP I and CRABP I are found in different cell compartments in the frontonasal area where retinoic acid has been implicated in olfactory pathway formation (Gustafson et al., 1993; LaMantia et al., 1993). The tight association of CRtBP and B-FABP expression and their structural homology may suggest that B-FABP is also implemented in the metabolism of a specific retinoid.

Alternatively, the ligand of B-FABP could be a fatty acid such as arachidonic acid or a related metabolite as anticipated from the structural homology with H-FABP (Grosse et al., 1992). More than 30 members of the nuclear hormone receptor family have been identified, including a number of ‘orphan’ receptors with yet unknown ligands (Green, 1993). The peroxisome proliferator activated receptor (PPAR) which belongs to this family can be activated by fatty acids (Keller et al., 1993). RXXR can be activated by eicosanoids (Eager et al., 1992). B-FABP could be involved in the metabolism of a ligand for a particular nuclear hormone receptor. Since B-FABP, in contrast to CRtBPs, is only expressed in the nervous system, it is conceivable that its ligand and the proposed corresponding nuclear hormone receptor also act in a tissue-specific manner.

In conclusion, the observed tissue restriction and cell specificity of B-FABP expression suggest the existence of a tightly regulated metabolism of hydrophobic ligands with functional implications in neuronal and glial cell differentiation. Note: After submission of this manuscript, the cDNA sequence of the clone GC9, now designated brain lipid binding protein (BLBP), was published (Feng et al., 1994) proving its identity with B-FABP.

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