Fatty Acid Binding Protein: Localization and Functional Significance in the Brain

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Long chain fatty acids are important nutrients for brain development and function. However, the molecular basis of their actions in the brain is still to be clarified. Fatty acid-binding proteins (FABPs) belong to the multigene family of the intracellular lipid-binding protein. FABPs bind to long chain fatty acids, being involved in the promotion of cellular uptake and transport of fatty acids, the targeting of fatty acids to specific metabolic pathways, and the regulation of gene expression. FABPs are widely expressed in mammalian tissues, with distinct expression patterns for the individual protein. Although FABPs have been implicated to serve as regulators in systemic cellular metabolic pathways, recent studies have demonstrated the ability of FABPs to regulate functions of the brain, one of the most fat-enriched tissues in the body. This review summarizes the localization of FABPs in the brain, and recent progress in elucidating the function of FABPs in the brain.

PUFAs; brain; gene knockout mice; astrocyte; neuron.

Fatty acids are important in general as substrates for energy supply, as building blocks of membrane lipids, as signaling molecules and as precursors for lipid mediators. In the brain, fatty acids are major structural components, and very high levels of fatty acids can be found in the neuronal membrane and the myelin sheath: It is estimated that 50% of the neuronal membrane is composed of fatty acids (Zerouga et al. 1991; Bourre et al. 1992; Rapoport 2005). In particular, long chain polyunsaturated fatty acids (PUFAs: \( \geq 20 \) carbon atoms and \( \geq 3 \) methylene-interrupted cis double bonds), e.g. docosahexaenoic acid (DHA) and arachidonic acid (AA), has been implicated as important nutrients for the human brain development and function (Gordon 1987, 1997; Hamosh and Salem 1998). PUFAs are synthesized by successive desaturation and elongation of the essential fatty acids such as linoleic acid (18C2, n-6) and \( \alpha \)-linolenic acid (18C3, n-3) from the diet, and are abundant in the brain. PUFAs make up 20% of brain dry weight, including about 6% for AA and 8% for DHA. DHA and AA are determinants of membrane fluidity, which is important for the efficacy of neurotransmitter-receptor interaction and transporters (Zerouga et al. 1991; Bourre et al. 1992; Rapoport 2005). AA is of special importance as a second messenger in signal transduction (Farooqui et al. 1997), and DHA modulates the expression and repression of a sizeable number of genes that are involved in energy metabolism in the brain (Kitajka et al. 2002, 2004). Recent epidemiological evidence suggests that dietary consumption of some PUFAs...
such as DHA and eicosapentanoic acid (EPA), commonly found in fish oil, may modify the risk for certain neuropsychiatric disorders. Furthermore, decreased blood levels of omega-3 fatty acids have been associated with several neuropsychiatric conditions, including attention deficit (hyperactivity) disorder, Alzheimer’s disease, schizophrenia and depression (Muskiet and Kemperman 2006).

Since long chain fatty acids are hydrophobic molecules, they are solubilized and transported within the cell to exert their cellular functions by specific intracellular lipid binding proteins, the low molecular mass polypeptides of 14~15 kDa termed fatty acid binding proteins (FABPs). FABPs have originally been named according to the tissue of their first isolation or of their prominent expression, e.g. liver-type (L-), intestinal-type (I-), heart-type (H-), brain-type (B-), epidermal-type (E-), and adipocyte-type (A-) (Gordon et al. 1983; Alpers et al. 1984; Hunt et al. 1986; Tweedie and Edwards 1989; Siegenthaler et al. 1993; Feng et al. 1994; Kurtz et al. 1994). However, a numerical nomenclature for the various FABP genes has recently been introduced and used widely, because they show much wider tissue distribution than originally thought (Iseki and Kondo 1990; Watanabe et al. 1991; Owada et al. 1996b, 2001, 2002a; Abdelwahab et al. 2003, 2007; Kitanaka et al. 2003, 2006; Yun et al. 2004; Nourani et al. 2005a, b). Several roles have so far been assigned to FABPs (Spener et al. 1989; Glatz et al. 1993; Coe and Bernlohr 1998): (a) control of cellular uptake of fatty acids and their subsequent metabolism, (b) intracellular compartmentation/storage of fatty acids, (c) modulation of intracellular signal transduction, (d) carriers of signaling fatty acids to the nuclear receptors such as PPARs (Fig. 1).

FABP of the brain was purified for the first time from the rodent brain as a protein fraction of low molecular weight capable of binding $[^{14}C]$-labeled oleic acid (Bass et al. 1984). This protein was later revealed to be identical to H-FABP (FABP3), and its mRNA expression increased from low levels in brains of late fetal to a subsequent 2- 3 fold levels in adult brain (Heuckeroth et al. 1987). Later studies revealed two further FABP-types, E-FABP (FABP5) and B-FABP (FABP7), to be expressed in the brain (Bennett et

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**Fig. 1.** Schematic presentation of the cellular FABP function.

The transmembrane uptake of fatty acids is facilitated by membrane-associated proteins, i.e., FATP and FAT(CD36). Intracellularly, fatty acids are bound by FABP, and FABP modulates various cellular processes including synthesis of acyl-CoA or eicosanoids, FA-mediated signal transduction and nuclear transcription by PPAR.

FA, fatty acid; FAT, fatty acid translocase (CD36); FATP, fatty acid transport protein; PPAR, peroxisome proliferator activated receptor; RA, retinoic acid; RXR, retinoid X receptor.
Our detailed histological studies have reported the localization of H-FABP, E-FABP and B-FABP in the developing and mature rodent brain under normal and pathological conditions (Owada et al. 1996a, b, 1997). Although these three species of FABP expressed in the brain all bind long-chain fatty acids (LCFAs), differences in preference for fatty acid binding have been noted as follows: H-FABP for n-6 polyunsaturated fatty acids, E-FABP for saturated fatty acids and B-FABP more for n-3 polyunsaturated fatty acids (Hanhoff et al. 2002). Therefore these three FABPs have been suggested to differentially modulate the actions of various LCFAs species on the brain function. Being aware that the brain has the highest heterogeneity in structure and function among various organs, we address in this review the localization of H-, E- and B- FABP, respectively, in the brain under normal and pathological conditions, and the reported functions of FABPs of the brain, which possibly link to their ability to bind LCFAs.

Localization of FABPs in the Rodent Brain

**H-FABP (FABP3)**

In our in situ hybridization analysis (Owada et al. 1996b), significant expression of H-FABP mRNA and protein is not observed in embryonic brains. After birth, H-FABP mRNA expression in brain becomes gradually positive with postnatal age, but is confined to the gray matter, the region in which neuronal cell bodies accumulate, throughout the postnatal course. At adult stage, distinct H-FABP expression is detected in the olfactory mitral cell layer, in the cerebral neocortex (layers II-VI) and in the hippocampal neuronal layers. In the cerebellum, H-FABP mRNA expression is evident in the Purkinje cells as well as the granule cells. H-FABP mRNA is not detected in the white matter, the ependymal cell layer, and the glia limitans delineating the external surface of rat brain (Table 1). In immunohistochemistry (Owada et al. 2006), H-FABP is detected in the neurons in the cerebral cortex and hippocampal pyramidal cells in the CA1 and CA2 portion.

Recently, H-FABP has been identified as an interacting molecule of dopamine D2 receptor using a yeast two hybrid system with mouse brain cDNA library, and H-FABP is co-localized to the D2 receptor-positive cells in the mouse brain (Takeuchi and Fukunaga 2003). With regard to the subcellular localization in the neuron, it has been reported that H-FABP is highly concentrated in synaptosomes from total brain of 4 months old mice but decreased thereafter (Pu et al. 1999).

**E-FABP (FABP5)**

Based on our analysis by Northern blot (Owada et al. 1996b), E-FABP mRNA is already expressed in rat brain at mid-term embryonic stages. After highest expression levels at late embryonic stages, E-FABP mRNA level decreases postnatally to become weak in the adult brain.

By in situ hybridization (Owada et al. 1996b), E-FABP is revealed to be expressed in the ventricular germinal zone as well as the cerebral cortex at the embryonic stage, and in the external granule cell layer, another germinal zone of early postnatal cerebellum. The gray matter throughout postnatal brain expresses E-FABP mRNA at weak to moderate levels. Among various gray matter regions, the expression is more evident in the cerebral cortex and the hippocampus. In the adult, the expression is positive in both neurons and glia, however, with varying intensity in various brain regions (Table 1). In immunohistochemistry, E-FABP is found in the hippocampal radial glia and astrocytes scattered throughout the brain, and in the neurons in the cerebral cortex (Liu et al. 2000; Owada et al. 2006).

An interesting feature of E-FABP expression is the inducibility under various pathological conditions: E-FABP expression is markedly enhanced in the neuron following peripheral nerve axonal injury (De Leon et al. 1996; Owada et al. 1997), suggesting a role in the regeneration of neurons. E-FABP mRNA, together with B-FABP mRNA, is also induced in the hippocampal astrocytes in the brain under kainic acid-induced seizure (Owada et al. 1996a).
In Northern blot analysis of mRNAs from rodent brains (Feng et al. 1994; Kurtz et al. 1994; Owada et al. 1996b), the gene encoding B-FABP is abundantly expressed at mid-term embryonic stages. As the differentiation of the embryonic brain progresses, B-FABP gene expression decreases gradually and becomes very weak in adult brains.

In embryonic rodent brain B-FABP mRNA is very intensely expressed in the radial glia (neural stem cells) in the ventricular and subventricular zones, where the neurogenesis is prominent. At neonatal stages, the expression in the germinal zones remains most intense, and it is strongly positive in both the gray and white matter throughout the neonatal brain. Among brain regions, the expression is more evident in the olfactory nerve fiber layers, hippocampal dentate gyrus, and the cerebellar Purkinje cell layer. The expression of B-FABP mRNA decreases markedly throughout the entire brain; however it remains clearly in the Schwann cells of olfactory nerve fiber layer, in the radial glia of dentate gyrus and in the Bergmann’s glia in the Purkinje cell layer (Table 1).

### Table 1. Summary of localization and function of three FABPs in the brain.

<table>
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<tr>
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<th>H-FABP (FABP3)</th>
<th>E-FABP (FABP5)</th>
<th>B-FABP (FABP7)</th>
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<tr>
<td><strong>Localization</strong></td>
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<td>Embryonic brain (E18)</td>
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<td>Cerebral cortex</td>
<td>n.d.</td>
<td>++ (VGZ&amp;N)</td>
<td>+++ (VGZ)</td>
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<td>Cerebellum</td>
<td>n.d.</td>
<td>+ (RG)</td>
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<td>Adult brain (P70)</td>
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<td>Olfactory bulb</td>
<td>+ (N)</td>
<td>+ (N)</td>
<td>++ (OEC)</td>
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<tr>
<td>Cerebral cortex</td>
<td>+ (N)</td>
<td>+ (N&amp;G)</td>
<td>+ (G)</td>
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<tr>
<td>Caudate putamen</td>
<td>+ (N)</td>
<td>n.d.</td>
<td>+ (G)</td>
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<td>Hippocampus</td>
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<td>CA1</td>
<td>+ (PC)</td>
<td>+ (PC&amp;G)</td>
<td>+ (G)</td>
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<td>CA2</td>
<td>++ (PC)</td>
<td>+ (PC&amp;G)</td>
<td>+ (G)</td>
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<td>CA3</td>
<td>+ (PC)</td>
<td>+ (PC&amp;G)</td>
<td>+ (G)</td>
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<td>Dentate gyrus</td>
<td>+ (G)</td>
<td>+ (GC, RG)</td>
<td>++ (RG)</td>
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<td>Thalamus</td>
<td>++ (N)</td>
<td>+ (N&amp;G)</td>
<td>+ (G)</td>
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<td>Hypothalamus</td>
<td>+ (N)</td>
<td>+ (N&amp;G)</td>
<td>+ (G)</td>
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<tr>
<td>Cerebellum</td>
<td>+ (GC)</td>
<td>+ (Pu&amp;RG)</td>
<td>++ (RG)</td>
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<td>White matter</td>
<td>n.d.</td>
<td>+ (G)</td>
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<td><strong>Function</strong></td>
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<td>Upatake of n-6 fatty acid into the brain,</td>
<td>NGF-dependent neurite outgrowth,</td>
<td>Emotional behavior, Schwann cell-axon interaction, Maintenance of neural progenitors, Schizophrenia endophenotype</td>
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<td>Emotional behavior</td>
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+++, intense; ++, moderate; +, weak; n.d., not detected.

G, glia; GC, granule cell; N, neuron; OEC, olfactory ensheathing cell; PC, pyramidal cell; Pu, Purkinje cell; RG, radial glia; VGZ, ventricular germinal zone.
In immunohistochemical studies on the localization of B-FABP in mouse embryonic and adult brain (Feng et al. 1994; Kurtz et al. 1994), many cells in the ventricular germinal zone of the brain and the spinal cord are intensely immunoreactive for B-FABP. Throughout the postnatal development to the adult stage, B-FABP is detected in astrocytes of the white matter, in radial glial cells spanning the granule cell layer of the dentate gyrus, and in Bergmann’s glial cells located adjacent to the cerebellar Purkinje cells.

With regard to the B-FABP expression in the pathologic condition, it has been reported that B-FABP expression is elevated in the Down syndrome brains (Sanchez-Font et al. 2003), suggesting that the overexpression of B-FABP could contribute to Down syndrome-associated neurological disorders. Very recently, we have reported the elevation of B-FABP mRNA expression in the prefrontal cortex of the brains from schizophrenic patients. (Watanabe et al. 2007).

**Functional Significance of FABPs in the Brain**

**H-FABP (FABP3)**

H-FABP-null mice exhibits the impaired uptake of fatty acids and their cellular utilization in the cardiac myocytes, where H-FABP is abundantly expressed (Binas et al. 1999). H-FABP comprises ~0.01% of the total cytosolic protein of the mouse brain (Myers-Payne et al. 1996), suggesting its involvement in uptake and metabolism of fatty acids in the neurons (Owada et al. 1996b). As expected, the incorporation of \[^{14}\text{C}]\text{AA (20:4 n-6)} into the brain reduces by 24% in the H-FABP-null mice, while that of \[^{14}\text{C}]\text{palmitic acid (16:0)} is unaffected (Murphy et al. 2005). Furthermore, in the brain of H-FABP-null mice, the proportion of total n-6 fatty acids is reduced in the major phospholipid classes (Murphy et al. 2005). Thus, H-FABP appears to have a similar role in the brain in terms of AA incorporation as it does in heart.

Although it should be elucidated in further studies how impaired uptake of n-6 fatty acid into the brain cells affects the neuronal functions in vivo, our preliminary studies shows that H-FABP-null mice exhibit the increased fear/anxiety like B-FABP-null mice (unpublished data). It is interesting to note that H-FABP interacts with a long isoform of dopamine D2 receptor (D2LR) which has been shown to be associated with several neuropsychiatric disorders (Takeuchi and Fukunaga 2003). Together with the fact that a deficiency of PUFA including DHA in the rodent is associated with a reduction in dopamine D2R binding density (Delion et al. 1996; Zimmer et al. 2000), H-FABP may modulate the emotional responses via the cellular uptake of fatty acid and the molecular interaction with dopamine D2R in the brain.

**E-FABP (FABP5)**

Induction of E-FABP expression following the peripheral nerve injury suggests its role in the regeneration of neurons (De Leon et al. 1996; Owada et al. 1997). Allen et al. (2001) have revealed that E-FABP expression is dependent on NGF in PC12 cells, and that E-FABP-deficient PC12 cells created by the antisense transfection decrease their NGF-dependent neurite outgrowth, suggesting that the induction of E-FABP during neurite outgrowth occurs for the purpose of mobilizing the fatty acid substrates necessary for the active synthesis of phospholipids and membranes.

E-FABP is supposed to be required for development of the brain, because its mRNA and protein are significantly detected throughout the brain developing stages. However, E-FABP-null mice generated by us are viable, and neither macro- nor microscopic abnormalities have been detected in the brain (Owada et al. 2002b). Further studies are necessary to clarify the physiological function of E-FABP in the brain.

**B-FABP (FABP7)**

The role of B-FABP in the neural cells has been investigated in vitro and/or in cells: Administration of anti B-FABP antibody to mixed cultures of cerebellar neurons and glia inhibits the formation of glial fascicles and migration of neurons along them, suggesting that B-FABP is released to serve extracellularly as a migrational cue for...
immature neurons (Feng et al. 1994), and Miller et al. (2003) also have reported that B-FABP-blocking antibodies enable the process outgrowth from Nf1-null TXF cells and restore the interaction with axons, suggesting possible roles of B-FABP to regulate Schwann cell-axon interactions.

It has been reported that knocking-down B-FABP expression by B-FABP small interfering RNA in cortical neuroepithelial cells impairs cell proliferation but promoted neuronal differentiation (Arai et al. 2005), suggesting the role for regulating the proliferation/differentiation of neural stem/progenitor cells during early cortical development. We have recently reported that B-FABP gene knockout mice shows altered emotional behavioral responses without marked abnormalities in the brain structure (Owada et al. 2006), suggesting that B-FABP deficiency during the brain development of B-FABP-null mice is mostly compensated by the other FABPs, possibly E- or H-FABP. Furthermore, we have very recently revealed that the neurogenesis in the dentate gyrus of B-FABP-null mice is attenuated, and B-FABP-deficient mice shows decreased prepulse inhibition (PPI), a biological marker for schizophrenia (Watanabe et al. 2007).

**CONCLUSION**

LCFAs, ligands of FABPs, are key players in many neural cell functions, including membrane remodeling, control of receptor activity and signal transduction. Therefore it is to be expected that FABPs, due to their ability of binding LCFAs with high affinity, participate in various neural cell functions. Existence of multiple FABP molecules with different biochemical features, e.g. H-, E- and B-FABP, in the brain suggests an adaptive pathway within the brain to regulate LCFA transport and metabolism to match the change of cellular needs for given LCFAs species, thereby being associated with their specialized brain functions. The demonstration of the phenotype of FABP-null mice may provide a broad overview of the physiological roles of each FABP in the brain, and a possible link between the molecular function and pathology of neuropsychological diseases like schizophrenia and bipolar disorder.

**Acknowledgments**

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**References**


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