Fatty Acid-binding Protein 5 (FABP5) Regulates Cognitive Function Both by Decreasing Anandamide Levels and by Activating the Nuclear Receptor Peroxisome Proliferator-activated Receptor β/δ (PPARβ/δ) in the Brain**

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Endocannabinoids modulate multiple behaviors, including learning and memory. We show that the endocannabinoid anandamide (AEA) can alter neuronal cell function both through its established role in activation of the G-protein-coupled receptor CB1, and by serving as a precursor for a potent agonist of the nuclear receptor PPARβ/δ, in turn up-regulating multiple cognition-associated genes. We show further that the fatty acid-binding protein FABP5 controls both of these functions in vivo. FABP5 both promotes the hydrolysis of AEA into arachidonic acid and thus reduces brain endocannabinoid levels, and directly shuttles arachidonic acid to the nucleus where it delivers it to PPARβ/δ, enabling its activation. In accordance, ablation of FABP5 in mice results in excess accumulation of AEA, abolishes PPARβ/δ activation in the brain, and markedly impairs hippocampus-based learning and memory. The data indicate that, by controlling anandamide disposition and activities, FABP5 plays a key role in regulating hippocampal cognitive function.

The intracellular lipid-binding protein (iLBP)† termed fatty acid-binding protein 5 (FABP5) is expressed during neurogenesis and in mature neurons in most regions of the brain (1–3). However, the nature of its role(s) in the brain and the exact CNS function(s) that rely on FABP5 are poorly understood. The functions of many members of the iLBP family remain to be identified. One exception is a group of iLBPs involved in regulation of gene transcription by cooperating with specific members of the nuclear receptor family transcription factors (4–9). Among these, FABP5 selectively delivers ligands from the cytosol to the nuclear receptor PPARβ/δ, and thereby it markedly enhances the transcriptional activity of the receptor (7, 8). It was indeed shown that the cooperation between FABP5 and PPARβ/δ underlies biological functions such as regulation of energy homeostasis (10) and promotion of prostate and mammary tumorigenesis (11, 12). Like FABP5, PPARβ/δ is expressed in most regions of the brain (13, 14), and it was reported that the FABP5/PPARβ/δ pathway inhibits differentiation of a cultured cell model of neuronal stem cells to neuronal progenitor cells, but is necessary for differentiation of progenitor cells to mature neurons (2). Whether FABP5 cooperates with PPARβ/δ in regulating neuronal differentiation or function in vivo is unknown, but the observations that a PPARβ/δ agonist enhances hippocampal neurogenesis and spatial memory in mice (15) raises the question of whether FABP5 is involved in regulating cognitive function.

An additional function for FABP5 was recently suggested by the observations that its overexpression in cultured neuroblastoma cells enhances cellular uptake of the endocannabinoid anandamide (arachidonoyl ethanolamide, AEA), and promotes hydrolysis of AEA into arachidonic acid (AA) and ethanolamine (16), a reaction catalyzed by fatty acid amide hydrolase (FAAH) (17). It was thus proposed that FABP5 delivers AEA to the enzyme (16). AEA activates the cannabinoid receptors CB1 and CB2, thereby triggering cellular signaling cascades (18) and modulating learning and memory (19–24). It is currently unknown whether AEA can activate PPARβ/δ (25), but it has been reported that AA can function as an agonist for this recep-
tor (26). Available information thus raises the intriguing possibility that endocannabinoids may affect brain function both by activating CB1/CB2, and either directly or through a metabolite, by activating PPARβ/δ. The work described here was carried out to study the involvement of FABP5 in these activities in vivo and to explore the functional consequences of such an involvement.

**EXPERIMENTAL PROCEDURES**

**Reagents and Plasmids**

Antibodies against FABP5, PPARβ/δ, actin, PDK1, and VEGFA were obtained from R&D Systems, Millipore, Santa Cruz Biotechnology, BD Transduction Laboratories, and Abcam, respectively. Antibodies against phospho-ERK, ERK1/2, and phospho-Šer-473-akt were purchased from Cell Signaling. Antibodies toward active Gαi and total Gαi were purchased from NewEast Biosciences (Malvern, PA). Horseradish peroxidase (HRP)-conjugated and Alexa Fluor 488-conjugated secondary antibodies were obtained from Bio-Rad and Invitrogen, respectively. AEA, AA, the FAAH inhibitor URB597, lipoxigenase-nas protease inhibitor nordihydroguaiaretic acid, cytochrome P450 epoxide inhibitor MS-PPOH, and the cyclooxygenase inhibitor sulindac were purchased from Cayman Chemical (Ann Arbor, MI). Oleic acid and GW0742 were purchased from Sigma and TorontoResearch Diagnostics, respectively. (PPRE)3-luciferase construct and expression vectors for PPARγ, PPARβ/δ, and PPARγ were previously described (8). Bacterial expression vector for histidine-tagged FABP5 was constructed into pcDNA3-His_MBP vector. Lentiviral plKO.1 vector harboring shRNA targeting mFABP5 (TRCN0000011894) or GFP (SHC003) was purchased from Open Biosystems and Sigma, respectively.

**Cell Culture**

N18TG2 mouse neuroblastoma cells (Sigma) were maintained in DMEM containing 10% fetal bovine serum at 37 °C in a humidified incubator with 5% CO2 and 95% air mixture. N18TG2 cells were plated at 90% confluency in 6-well plates, washed twice in DMEM, and incubated for 10 min at 37 °C with 750 μl of [14C]AEa (100 nM) that was pre-equilibrated for 75 min in DMEM supplemented with 0.15% BSA. Control plates were incubated on ice. Media were collected, and the cells were washed with cold BSA-supplemented medium and lysed in ice-cold PBS containing 2 mM EDTA (400 μl). 3 ml of chloroform/methanol (1:1) were added to the media, and 2.4 ml of chloroform/methanol (1:1) were added to the cells followed by phase separation by centrifugation (2000 rpm, 10 min). The resultant aqueous (containing [14C]ethanolamine) and organic (containing [14C]AEa) phases of cell and media were counted by liquid scintillation counter. [14C]AEa uptake was determined by
summing the amounts of $[^{14}C] $ethanolamine in the media and cells with intact cellular $[^{14}C] $AEA. Hydrolysis of $[^{14}C] $AEA was quantified by subtracting $[^{14}C] $ethanolamine in the media and cells of the control plates.

**Measurements of AEA in Mouse Brain**

Whole brains were collected from 12–14-week-old FABP5$^{-/-}$ males and WT littermates. AEA concentrations were measured as described in Ref. 28.

**Bacterial Expression of FABP5**

Histidine-tagged FABP5 was expressed in the *Escherichia coli* strain BL21. Bacteria were grown in LB medium at 37 °C to an $A_{600 \text{ nm}}$ of 0.8. Protein expression was induced by 1 mm isopropyl-1-thio-β-D-galactopyranoside followed by an additional overnight incubation at 15 °C. Bacteria pellets were homogenized in B-PER extraction reagent (Thermo Scientific). His-tagged FABP5 was loaded on a nickel-charged Sepharose column (nickel/SePhorose 6 Fast Flow, GE HealthCare) and washed with Buffer A (25 mm imidazole, 300 mm NaCl, 100 mm Tris, pH 7.4, 5% glycerol) and 5% buffer B (250 mm imidazole, 300 mm NaCl, 100 mm Tris, pH 7.4, 5% glycerol). The protein was eluted with 25% buffer B and dialyzed against buffer containing 300 mm NaCl, 100 mm Tris, pH 7.4, and 5% glycerol. Protein concentration was measured by protein absorbance using $ε_{280 \text{ nm}} = 13,980 \text{ M}^{-1}\text{cm}^{-1}$.

**Transactivation Assays**

N18TG2 cells were cultured in 6-well plates and co-transfected with PPRE-luciferase reporter vector, an expression vector for PPARβ/δ, PPARα, or PPARγ, and a vector harboring β-galactosidase, serving as an internal control. 18 h after transfection, cells were incubated in DMEM supplemented with 10% charcoal-treated FBS and then treated with a ligand for additional 18 h. Cells were lysed, and luciferase activity was assayed using luciferase assay buffer (Promega, WI) and corrected for transfection efficiency by the activity of β-galactosidase.

**Confocal Fluorescence Microscopy**

Cells, cultured in DMEM supplemented with 10% delipidated FBS, were washed with cold PBS and fixed in 4% paraformaldehyde (15 min). Cells were permeabilized using PBS containing 0.2% Triton X-100 and blocked using PBS supplemented with 10% donkey serum. FABP5 was immunostained with goat anti-mouse FABP5 antibody (1:100 dilution) overnight at 4 °C. Cells were stained with Alexa Fluor 488-labeled donkey anti-goat IgG secondary antibody, and nuclei were visualized by DAPI staining. Confocal fluorescence imaging was performed using Zeiss LSM510 confocal microscope.

**Microarray Experiment and Data Analysis**

N4 cells, which highly express FABP5 (12), were cultured in medium supplemented with 10% charcoal-treated FBS for 48 h and then treated with vehicle or GW0742 (1 μM) for 4 h. Total RNA was extracted by QIAzol (Qiagen) and purified using RNeasy columns (Qiagen). Samples were amplified, labeled, and hybridized on Affymetrix® Mouse Gene 2.1 ST arrays (Affymetrix) by the Gene Expression and Genotyping Facility of the Case Comprehensive Cancer Center of Case Western Reserve University. Raw data files were analyzed using Partek-Genomics-Suite (PGS) v6.6 software. Data were normalized using the Robust Multichip Average Method (RMA), which allows reduction of block effect done at the probe-set level. Student’s t test analysis was used to select differentially expressed genes with -fold change and $p$ value cutoffs respectively fixed to 1.5 and 0.05. The network of genes with known functions in behavior, neuronal development and function, and molecular transport was identified using IPA (Ingenuity Systems) ($p < 0.002$).

**Behavioral Procedures**

**T-maze**—Mice were placed in a clear plexiglass T-maze (with arms 60 cm of length) and were allowed to explore the maze freely for 10 min while one of the arms was blocked. The blocked arm was switched between animals to avoid any arm preference bias (counterbalanced). Following a 10-min exploration, mice were returned to their home cage for 3 h and then put back in the T-maze, this time, with all three arms open. Once put in the T-maze, mice were video recorded using EthoVision tracking system, and the first arm choice, time and frequency in the previously blocked arm, and total numbers of arm entries were counted by using video-scoring software.

**Fear Conditioning**—All animals were placed in a conditioning box (Med Associates, Burlington, VT) and trained to associate a tone (80 dB for 30 s.) with electrical shock (0.5 mA for 0.5 s.). This procedure was repeated five times over a course of 2 min. A delay of 1.5 s between tone and shock was introduced as a trace in making the task more cognitively demanding. At the end of the trial, the animals were taken out and placed back in the box 24 h later to evaluate their learned aversion for an environment associated with the shock (context-dependent fear). To this end, all animals were placed in a box in which they were trained for the duration of 5 min, and freezing behavior in the absence of tone or aversive stimulus were measured. The animals were then removed, and the context was changed so that the animals could no longer recognize the chamber in which they had been trained. 2 h after the animals were tested for contextual fear conditioning, they were reintroduced into the new contextually altered box, and freezing behavior was measured during the first 3 min to verify that the animals did not recognize the context. After 3 min, the tone was delivered, and freezing behavior was measured to determine cue-dependent fear conditioning for the following 3 min.

**Morris Water Maze**—The spatial version of the water maze task was used to examine cognitive deficits following a modified version of previously published methodology (29). Briefly, animals were trained in a black circular pool (120 cm), in a well lighted room replete of visual cues. Pool water was whitened with nontoxic white dye and temperature was maintained at 23 °C. A clear escape platform (10.5 cm in diameter) was located ~0.5 cm beneath the water level placed in the center of a quadrant (N, S, E, W) of the pool in the same location relative to room visual cues. Animals were tested for eight trials per day, subdivided into two blocks of four trials, over 5 days. Prior to the beginning of testing, the animals were allowed to swim freely in the pool for 30 s and then allowed to sit on the escape
platform for an additional 30 s. On days 1–5 of testing, the platform was located in quadrant 4 (northwest quadrant) for all eight trials. The animals were placed in the water from one of the four start positions at the edge of each quadrant and allowed to swim for 60 s. If the animal did not find the platform during the allocated task, it was guided toward it, and after 15 s on the platform, it was placed back into the water from the next start position. The exact procedure was followed for four trials (one from each start position), at which point the mouse was dried and placed back into its home cage (warmed with a heating lamp) for 30–40 min until the start of the next trial block. Swim time, path length, and swim speed were recorded using a tracking system and software (EthoVision, Noldus). On day 5, the platform was placed in the same position as day 1 for the seven trials and removed on the eighth trial for a probe trial to test for spatial strategy and retention. During this trial, the platform was removed, and the animals were allowed to swim for 60 s without the possibility to escape; the percentage of time spent in the quadrant where the platform was previously located, proximity (average distance to the platform in cm), and platform crossings (the number of times the animal crosses the exact location) in addition to latency to enter the target quadrant were measured. On day 6, animals were tested for visual acuity by rendering the platform visible and removing all extramaze cues. Eight trials in two four-trial blocks were administered with the platform moving to a different quadrant for each trial. Animals with average escape latencies higher than 30 s after eight trials were considered visually impaired and excluded from the analyses.

**Statistical Analyses**

Morris water maze test data for training (learning) was analyzed using a one-way analysis of variance with a within-subjects repeated measures factor of day of training. Probe performance was analyzed using an independent sample Student’s t test. T-maze and fear conditioning differences for all variables measured were analyzed using independent sample Student’s t tests.

**RESULTS**

**FABP5 Is Required for Activation of PPARβ/δ in Brain**—Transgenic reporter mice in which luciferase expression is under the control of a PPAR-inducible promoter in all target organs (PPRE-luc) (27) were used to examine whether FABP5 cooperates with PPARβ/δ in mouse brain in vivo. PPRE-luc mice were crossed with FABP5-null mice (30) (Fig. 1a) to obtain PPRE-luc+/−/FABP5−/− and PPRE-luc+/−/FABP5+/− littermates. Mice were injected with luciferin and euthanized 10 min later, and whole brains were harvested and imaged. The observations strikingly revealed that although PPAR is highly activated in brains of WT mice, this activity is all but abolished in brains of FABP5−/− animals (Fig. 1b). In principle, the luciferase reporter may be activated by all PPAR isotypes. However, the observations that FABP5 is critical for the activation indicate that the predominant PPAR activity in the brain emanates from PPARβ/δ, the cognate FABP5 receptor (7, 8) (see Fig. 4, d–g). As the expression level of PPARβ/δ itself was not altered in FABP5−/− brains (Fig. 1c), the data demonstrate that FABP5 is critical for activation of the receptor in this tissue. In agreement with suppression of receptor activity, levels of mRNA and protein of two well established direct PPARβ/δ target genes, *Pdpk1* and *Vegfa*, were distinctly lower in brains of FABP5−/− versus WT mice (Fig. 1, d–g).

**FABP5 Regulates the Level of AEA in Mouse Brain**—The signature activity of AEA is its ability to activate CB1, resulting in activation of Gαi, and downstream signaling cascades, includ-
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FIGURE 2. FABP5 promotes cellular uptake and hydrolysis of AEA and regulates its level in the brain. a, N18TG2 cells were treated with AEA (10 μM, 60 min), and active Gαi was immunoprecipitated (IP) and visualized by immunoblots. Its level was 20% of total lyase (20% Input) and the heavy chain of the antibody (IgG heavy chain) are shown as controls for total expression of Gαi and precipitation efficiency, respectively. b, N18TG2 cells were treated with AEA (5 μM), and the phosphorylation level of ERK (pERK) at denoted times was monitored by immunoblots. c, N18TG2 cells were treated with denoted concentrations of AEA for 5 min, and the phosphorylation level of ERK was monitored by immunoblots. d, levels of FABP5 mRNA in N18TG2 cell lines expressing different levels of FABP5, measured by Q-PCR. Inset, immunoblots of FABP5 in N18TG2 cell lines expressing different levels of FABP5. e, f, rates of uptake (e) and hydrolysis (f) of AEA in N18TG2 lines stably expressing varying levels of FABP5. See “Experimental Procedures” for details. Data are mean ± S.D. (n = 3). **p < 0.01. g, levels of mRNA for FABP3, FABP5, and FABP7 in whole brains of WT and FABP5−/− mice, measured by Q-PCR. Data are mean ± S.D. n = 3. h, levels of AEA in whole brains of 12–14-week-old FABP5−/− male mice and WT littermates. n = 10/group. See “Experimental Procedures” for details. i, left, levels of active Gαi protein in hippocampus of two WT and two FABP5−/− mice, assessed by immunoblots following immunoprecipitation of active Gαi. Right, quantification of immunoblots. j, immunoblots of phospho-ERK (pERK) and phospho-AKT (pAKT) in hippocampus of two WT and two FABP5−/− mice.

Mouse brain expresses FABP3, FABP5, and FABP7 (36). Expression of FABP7 and FABP3 was not significantly altered in brains of FABP5-null mice (Fig. 2g), indicating that these FABPs do not compensate for FABP5 deficiency and suggesting that the protein has a unique function in the brain. In keeping with a role for FABP5 in promoting AEA degradation in the brain, the level of the compound was found to be ~50% higher in brains of FABP5−/− versus WT mice (Fig. 2h). Accordingly, the activation status of Gαi (Fig. 2i) and the phosphorylation level of two downstream effectors of the CB1 pathway, ERK and AKT (Fig. 2j) were significantly higher in hippocampus of FABP5−/− versus WT mice.

PPARβ/δ Is Activated by the AEA Metabolite AA but Not by the Endocannabinoid Itself—The observations that FABP5 is required for activation of PPARβ/δ in the brain suggest that it mediates nuclear transport of an important PPARβ/δ ligand in this tissue. The ligand selectivity of PPARβ/δ is quite broad, and it can be activated by multiple lipid substances including various unsaturated fatty acids and the vitamin A metabolite retinoic acid (RA) (7, 37–39). Possibly, either AEA itself or its metabolite AA may function as agonists for this receptor. To examine whether either of these compounds can activate PPARβ/δ, their effects on expression of the direct PPARβ/δ target genes Plin2 (40), Pdpk1 (41), and Ascl6 (42) in N18TG2 cells were examined. The data show that both compounds induced the expression of these genes with AA displaying a somewhat better efficacy than AEA (Fig. 3, a–c). Transcriptional activation assays were carried out to further investigate whether these ligands can activate PPARβ/δ. In these assays, to ensure that observed effects specifically reflect activation of PPARβ/δ and not other PPAR subtypes, N18TG2 cells were transfected both with a vector encoding a luciferase reporter gene driven by a PPRE and with an expression vector for PPARβ/δ. Cells were treated with the synthetic PPARβ/δ-selective ligand GW0742, AEA, or AA, and luciferase activity was measured. Both AEA and AA activated the expression of the PPARβ/δ-driven reporter (Fig. 3d). However, although pre-treatment of the cells with the FAAH inhibitor URB597 did not affect the activity of either the GW0742 or the AA, it completely abolished the activity of AEA (Fig. 3e). These observations indicate that AEA does not directly activate PPARβ/δ, but rather, serves as a precursor for such a ligand, and that generation of the ligand requires FAAH activity. A mixture of inhibitors of enzymes that catalyze AA metabolism was then used to exam-

ing the MAPK/ERK pathway (18, 31–35). Indeed, treatment of N18TG2 neuroblastoma cells, which express CB1, with AEA resulted in activation of Gαi (Fig. 2a) and in phosphorylation of ERK (Fig. 2b and c). It has been reported that ectopic overexpression of FABP5 in N18TG2 cells potentiates the uptake of AEA and facilitates its degradation (16). To further examine the role of FABP5 in cellular handling of AEA, two cell lines that stably express different levels of the protein (sh3 and sh4) were generated (Fig. 2d). Measurements of cellular uptake (Fig. 2e) and hydrolysis (Fig. 2f) of AEA revealed that reducing the expression of FABP5 markedly and in a dose-responsive manner inhibited both processes.
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FABP5 Is Mobilized to the Nucleus upon Binding AA and Is Essential for Enabling Activation of PPARβ/δ by This Ligand—FABP5 can bind a variety of ligands with a similar affinity, but it translocates to the nucleus only in specific response to compounds that activate its cognate nuclear receptor PPARβ/δ (7). To examine whether FABP5 can bind either AEA or AA, the protein was expressed in E. coli and purified (Fig. 3g), and the equilibrium dissociation constants (K_d) for its association with AEA and AA were measured by fluorescence competition assays. The method entails two steps (43). In the first step, the K_d for association of FABP5 with the fluorescent lipid probe anilinonaphtalene-8-sulfonic acid (ANS) was measured by fluorescence titrations. Titration with ANS resulted in a saturable increase in fluorescence (Fig. 3h), and analysis of titration curves (44) yielded a K_d of 70 ± 6.4 nM. K_d values for nonfluorescent ligands were then measured by monitoring their ability to compete with the probe for binding. FABP5 was precomplexed with ANS and titrated with ligands. Ligand binding was followed by monitoring the decrease in ANS fluorescence. Titration curve was fit (solid line through data points) to obtain the K_d, FABP5 was precomplexed with ANS and a 1:1 mole ratio and titrated with AEA or AA. Binding was monitored by following the decrease in ANS fluorescence.

Confocal fluorescence microscopy was used to examine whether either AEA or AA can activate the nuclear translocation of FABP5. N18TG2 cells were cultured in charcoal-treated medium to deplete FABP5 ligands. Cells were then treated with GW0742, AEA, or AA for 40 min, immunostained using FABP5 antibodies, and imaged. The data (Fig. 4, a and b) show that FABP5 was excluded from the nucleus in untreated cells and that GW0742, AEA, and AA, but not the other FAAH product ethanolamine, induced the protein to undergo nuclear translocation. Similarly, both AEA and AA induced mobilization of FABP5 to the nucleus in a derivative of NaF mammary carcinoma cells, a line that expresses a high level of this protein (7) (Fig. 4c). Pretreatment with the FAAH inhibitor URB597 pre-...
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FIGURE 4. FABP5 is mobilized to the nucleus upon binding AA and is essential for activation of PPARβ/δ by this ligand. a, N18TG2 cells, cultured in delipidated medium, were treated with GW0742, AEA, AA, or ethanolamine (10 μM, 40 min) in the absence or presence of the FAAH inhibitor URB597 (URB, 100 nM) or a mixture of inhibitors of AA metabolism containing 5 μM nordihydroguaiaretic acid, 5 μM MS-PPOH, and 50 μM sulindac (inh). Cells were imaged using a Zeiss LSM510 confocal microscope. Scale bar = 10 μm. b, quantification of nucleus/cytosol FABP5 fluorescence intensity in cells treated as denoted. Data are mean ± S.D. n = 10–15 cells per treatment group. p values for differences between treated and untreated cells were: GW0742, *, p = 7E-5; AEA, **, p = 0.008; AA, ***, p = 0.0004. c, cells derived from NaF mammary carcinomas were cultured in delipidated medium and treated with AEA or AA (10 μM, 40 min). FABP5 (green) was immunostained, and nuclei were visualized by DAPI (blue). Cells were imaged by confocal fluorescence microscopy. Scale bar = 5 μm. d–g, transactivation assays were carried out using N18TG2 lines with varying levels of FABP5 expression (Fig. 2d). Cells were co-transfected with vectors encoding PPARβ/δ (d and e), PPARα (f), or PPARγ (g) together with a luciferase reporter driven by a (PPRE), and a vector harboring β-galactosidase. Cells were treated with GW0742 (GW; 1 μM), oleic acid (OA), or AA at the denoted concentrations. Data in d–g are mean ± S.D. (n = 4), **, p < 0.01 versus untreated controls. shGFP, shRNA targeting GFP. shF5-3 and shF5-4: two clones stably expressing FABP5shRNA (see Fig. 2d).

vented the ability of AEA to activate the nuclear translocation of FABP5, and AA retained its ability to mobilize FABP5 to the nucleus even in the presence of a mixture of inhibitors of its metabolism (Fig. 4a). The data thus indicate that AA directly activates the nuclear localization of FABP5 and that AEA exerts such an effect indirectly, by giving rise to AA.

To examine whether FABP5 is essential for AA-induced activation of PPARβ/δ, transactivation assays were carried out in N18TG2 cell lines that stably express varying levels of FABP5 (Fig. 2d). The data show that, in a dose-responsive manner, down-regulation of FABP5 decreased the basal activation level and abolished the ability of both the synthetic ligand GW0742 and AA to activate the PPRE-driven reporter (Fig. 4d). Activation of PPARβ/δ by the pan-PPAR ligand oleic acid was similarly suppressed upon decreasing FABP5 expression (Fig. 4e). It has been reported that AA can function as an agonist for the other PPAR isotypes, PPARα and PPARγ (45–47). To examine whether FABP5 also cooperates with these PPARs, either PPARα or PPARγ was ectopically overexpressed in cells that express different levels of FABP5, and transactivation assays were carried out (Fig. 4f and g). Both the pan-PPAR ligand oleic acid and AA activated PPARα as well as PPARγ, but down-regulation of FABP5 had no effect on the activity of either of these PPAR subtypes. The data thus indicate that FABP5 selectively cooperates with PPARβ/δ in mediating the transcriptional activity of its ligands, including AA.

The FABP5/PPARβ/δ Pathway Induces the Expression of Cognition-associated Genes—It has been reported that administration of PPARβ/δ agonists improves learning and memory in mice (15). However, the mechanism by which PPARβ/δ regulates cognitive function and the identities of genes that mediate this activity are unknown. To begin to identify such genes, the effects of the PPARβ/δ-selective agonist GW0742 on gene expression were examined in NaF cells, which express a high level of FABP5. Affymetrix transcriptome analysis revealed that multiple genes known to be involved in neuronal function were up-regulated following a 4-h treatment with GW0742, a time frame in which responsive genes are expected to predominantly comprise direct targets for the receptor (Fig. 5a). Among these, Pik3c2a (48) and Rock2 (49) are known to be associated with learning and memory. In accordance with their identification as targets for the FABP5/PPARβ/δ pathway, the expression levels of these genes were significantly lower in hippocampi of FABP5−/− versus WT mice (Fig. 5, b and c).

FABP5-null Mice Display Impaired Cognitive Function—In mouse brain, FABP5 is expressed in brain stem, midbrain, thalamus, hippocampus, and cortex, and at a somewhat lower level in cerebellum and olfactory bulb (Fig. 6a). Its high expression...
level in the hippocampus and the previous reports that ablation of FABP5 results in dysregulation of neurogenesis in this region (1, 2) raise the possibility that the protein may be involved in hippocampus-dependent cognitive function. Brains of FABP5−/− and WT mice were similar in size and weight, and H&E staining of brain sections did not show discernible morphological or structural differences. A behavioral phenotyping screen did not reveal differences between WT and FABP5−/− mice in assessments of anxiety, activity, pain sensitivity, motor control, coordination, or visual function. However, FABP5−/− mice showed significant impairment in learning and memory. Specifically, in a delayed-alternation version of the T-maze test, which examines short term spatial memory, FABP5−/− mice spent significantly less time exploring a previously blocked arm (Fig. 6b). This difference was more prominent during the initial exploration phase while displaying similar levels of arm explorations. In a fear conditioning test, which evaluates hippocampal and amygdalar dependent associative learning, FABP5−/− mice showed significant lower levels of freezing than WT mice in the context-dependent (hippocampal) associative memory (Fig. 6c, left panel) but not in cued-dependent (amygdalar) memory (Fig. 6c, right panel). Deficit in spatial learning in FABP5−/− mice was also detected using the Morris water maze test. These mice displayed a significant impairment in learning this task as shown by significantly increased escape latencies when compared with WT counterparts (Fig. 6d, left panel). Notably, neither swimming speed nor vision was different between the groups, indicating that the impairment is specific to learning and memory. Spatial strategy and retention of learned information were assessed using a probe trial that showed that WT mice had higher preference for the quadrant that had previously contained the platform when compared

FIGURE 5. FABP5 regulates expression of cognition-associated genes. a, bioinformatically predicted neuronal development and neuronal disorders gene network (p = 3.67 × 10−5) that was altered by with GW0742 treatment in NaF cells. Arrows indicate activation. Lines ending in short perpendicular lines indicate repression. Red shapes and green shapes indicate up-regulated and down-regulated genes, respectively. b and c, levels of Pik3c2a and Rock2 mRNA in hippocampus of FABP5−/− and WT mice, assessed by Q-PCR. Data are mean ± S.D. n = 8/group.
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with FABP5<sup>−/−</sup> mice (53 versus 32%, Fig. 6d, right panel). These differences did not reach statistical significance with the number of animals used, but the strong trend (p = 0.06) indicates memory impairment in the mutant mice.

**DISCUSSION**

The observations demonstrate that FABP5 closely regulates the fate and biological activities of endocannabinoids in vivo and that it does so through two distinct functions (Fig. 7). On the one hand, it promotes cellular uptake and hydrolysis of AEA. Although no direct evidence in conclusive support of this notion currently exists, these observations likely reflect that FABP5 directly delivers AEA to its metabolizing enzyme FAAH, located in endoplasmic reticulum membranes (16). On the other hand, upon binding AA, a FAAH-generated AEA metabolite, FABP5 mobilizes to the nucleus where it “channels” this ligand to PPARβ/δ, resulting in induction of PPARβ/δ target genes. The data suggest that AEA can affect cellular function both by triggering CB1-mediated signaling and by serving as a precursor for a potent PPARβ/δ agonist, and both functions are controlled by FABP5. Considering the profound effects of activation of CB1 on cognitive functions (19–24, 50, 51) and the involvement of PPARβ/δ in regulating neurogenesis in hippocampus and other neuronal functions (1,2,15) (Fig. 5), the observations raise the possibility that FABP5 plays a key role in hippocampus-associated function. Specifically, the data suggest that the ability of FABP5 to both prevent excess accumulation of endocannabinoids in the brain (Fig. 2h) and induce the expression of cognition-associated PPARβ/δ target genes (Fig. 5) will allow this protein to potently promote learning and memory. On the other side of this coin, enhanced activation of CB1 (Fig. 2, i and j) and near elimination of PPARβ/δ activation (Fig. 1b) in brains of mice lacking FABP5 can be expected to undermine cognitive function. Indeed, a behavioral phenotyping screen revealed that FABP5<sup>−/−</sup> mice display pronounced impairment in hippocampal spatial memory and that they show lower responses in context-dependent associative memory, a hippocampus-specific function (Fig. 6). The relative contributions of CB1 and PPARβ/δ to the observed phenotype remain to be determined.

It has been reported that, in cultured cells, both FABP5 and its isotype FABP7 can promote cellular uptake and hydrolysis of AEA (16). However, the observations that expression of FABP7 is not altered upon ablation of FABP5 (Fig. 2g) and that endocannabinoid levels and the activation status of the CB1 signaling are markedly higher in brains of mice lacking FABP5 indicate that, in vivo, FABP7 does not compensate for loss of FABP5 in regard to AEA processing. The observations that the activation of PPARβ/δ is all but abolished in brains of FABP5<sup>−/−</sup> mice further indicate that the cooperation of FABP5 with its cognate receptor is a specific feature, not shared by other FABPs. Moreover, unlike FABP5<sup>−/−</sup> mice characterized here, it was reported that mice lacking FABP7 do not display any deficit in memory impairment in the mutant mice.
in spatial learning/memory, but rather, they exhibit enhanced anxiety and increased fear memory (52). Hence, in addition to their distinct spatio-temporal expression patterns during specific developmental stages and processes in the brain (3), FABP5 and FABP7 have distinct biological roles.

iLBPs that cooperate with nuclear receptors, including FABP5, possess a nuclear localization signal that is activated upon binding of specific ligands that can trigger the transcriptional activity of their cognate receptors. This signal is composed of three basic residues that, although not adjacent in the primary sequence, form a classical nuclear localization signal motif in the three-dimensional fold of the proteins. Binding of agonists for their cognate receptors induces these iLBPs to undergo conformational changes that place these residues at a configuration recognized by importin α, which then carries the proteins to the nucleus (53, 54). Hence, although FABPs, including FABP5, bind multiple hydrophobic compounds with similar affinities, only specific compounds direct them to the nucleus (7).

These observations raise the question of the functional significance of the tight association of these iLBPs with ligands that do not activate their nuclear localization signal. The data presented here strikingly suggest that FABP5 is directed to FAAH when in complex with AEA, but is targeted to nuclear PPARβ/δ when associated with AA. It is tempting to speculate that the two functions of FABP5 are sequentially connected. An intriguing scenario is that FABP5 cooperates with FAAH both by directly “channeling” AEA to the enzyme and by facilitating the removal of the product from the catalytic site, thereby promoting catalysis. Following binding of AA, FABP5 dissociates from the enzyme and shuttles the fatty acid to the nucleus where it channels it to PPARβ/δ. The structural features of FABP5 that underlie its ligand-specific targeting to different subcellular sites and thus enable it to carry out multiple functions remain to be clarified.

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