Mol Neurobiol (2012) 45:144–152
DOI 10.1007/s12035-011-8227-8

Novel Dopamine D2 Receptor Signaling through Proteins Interacting with the Third Cytoplasmic Loop

Kohji Fukunaga · Norifumi Shioda

Received: 11 October 2011 / Accepted: 7 December 2011 / Published online: 20 December 2011

Abstract The diverse activities of dopamine D2-like receptors, including D2, D3, and D4 receptors, are mediated by proteins that interact with the third cytoplasmic loop and regulate receptor signaling, receptor trafficking, and apoptosis. Such interacting proteins include calmodulin, the *N*-methyl-D-aspartate receptor 2B subunit, calcium/calmodulin-dependent protein kinase II, prostate apoptosis response-4, and β-arrestins, which regulate receptor signaling and the pharmacological action through D2 receptor. The gene encoding the D2 receptor gives rise to two isoforms, termed the dopamine D2 receptor long isoform (D2L) and the dopamine D2 receptor short isoform; the latter lacks 29 amino acids of the D2L receptor within the third cytoplasmic loop. In this review, we first focus on novel functions of the hetero-oligomeric D1/D2 and D2/adenosine A_{2A} receptors. We next discuss novel signaling through proteins interacting with the D2 receptor third cytoplasmic loop and define the function of a novel binding protein, heart-type fatty acid binding protein, which interacts with the D2L third cytoplasmic loop.

Keywords Dopamine D2 receptor · Hetero-oligomeric D2 receptor · Heart-type fatty acid binding protein · CaMKII

Abbreviations

ACh	Acetylcholine
CaM	Calmodulin
CaMKII	Calcium/calmodulin-dependent protein kinase II
DAT	Dopamine transporter
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
Glu	Glutamate
H-FABP	Heart-type fatty acid binding protein
LCPUFA	Long chain polyunsaturated fatty acid
LTP	Long-term potentiation
MSN	Medium-sized spiny neuron
NMDA	*N*-methyl-D-aspartate
NR2B	NMDA receptor 2B subunit
NSF	*N*-ethylmaleimide-sensitive factor
Par-4	Prostate apoptosis response-4

K. Fukunaga (✉) · N. Shioda
Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Tohoku University,
Aramaki-Aoba Aoba-ku,
Sendai 980-8578, Japan
e-mail: fukunaga@mail.pharm.tohoku.ac.jp

Introduction

In the central nervous system, dopamine controls psychomotor functions such as locomotion, cognition, and emotion through diverse dopamine receptors. Dysregulation of dopamine release and receptor activity account for neuropsychotic disorders such as Parkinson's disease and schizophrenia. Dopamine receptors are members of the seven transmembrane and trimeric GTP-binding protein-coupled receptor (GPCR) family and are classified into distinct subfamilies, namely, dopamine D1-like (D1 and D5) and dopamine D2-like (D2, D3, and D4), based on pharmacological properties, sequence homology, and structure [1]. D1-like receptors exhibit short third cytoplasmic loops and very long carboxyl-terminal intracellular tails, while D2-like receptors exhibit long third intracellular loops and short carboxyl-terminal intracellular tails. D1-like receptors activate adenylyl cyclase by coupling to Gs protein. By contrast, D2-like receptors are coupled to pertussis toxin-sensitive $G_{i/o}$ proteins and signal through several pathways,

such as inhibition of adenylyl cyclase, activation of K^+ channels, inhibition of Ca^{2+} channels, or stimulation of Na^+/H^+ anti-porters [2]. However, D1 and D2 receptor signaling is complicated by the existence of hetero-oligomeric receptors such as D1/D2 and D5/D2 receptors, both of which account for diverse calcium signaling [3, 4]. Activation of D1/D2 hetero-oligomer receptors expressed in HEK-293 T cells is coupled with $G_{q/11}$ protein, thereby activating a phospholipase C-mediated calcium signal. Expression of D5 receptor alone promotes marked elevation of intracellular calcium levels from intracellular and extracellular compartments. Activation of D5/D2 hetero-oligomer receptors, however, suppresses D5 receptor-stimulated intracellular calcium mobilization [4]. D2 receptors also signal through the extensively studied extracellular signal-regulated kinase (ERK) pathway through transactivation of the platelet-derived growth factor (PDGF) receptor, which is also coupled to $G_{i/o}$ protein [5].

Among dopamine receptor subtypes, D2 receptor is the most extensively studied, since all antipsychotic drugs used clinically act as D2 receptor antagonists in the mesolimbic dopamine system, and the ability of these drugs to block D2 receptor closely correlates with antipsychotic therapeutic action in schizophrenia. The D2 receptor exists as two alternatively spliced isoforms—the long isoform (D2L) and the short isoform (D2S)—which differ in a 29 amino acid insert in the third cytoplasmic loop [6]. Human D2 receptors reportedly have a polymorphism changing serine to cysteine at amino acid 311 of the D2L third cytoplasmic loop [7]. That variant is reportedly associated with schizophrenia [8], although studies have not yet confirmed these reports [9]. Pharmacologically, the human D2 receptor Ser311Cys variant has been shown to be less effective in inhibiting cyclic AMP accumulation than is the wild-type D2 receptor when expressed in Chinese hamster ovary cells [10]. A recent study characterized a single nucleotide polymorphism within the D2 receptor gene in intron 6, rs1076560 (G>T), in postmortem human striatum and prefrontal cortex [11]. Compared to the GG genotype in humans, the GT genotype is associated with relatively lower expression of D2S receptor mRNA in prefrontal cortex and striatum, as well as with significantly reduced performance on a cognitive task [11].

In this review, we first document the pathophysiological relevance of calcium and ERK signaling through the D2 receptor and its hetero-oligomerization with other GPCRs such as D1, D5, and adenosine A_{2A} receptors. We then summarize the modulatory roles of proteins interacting with the third cytoplasmic loop of the D2 receptor in brain. Understanding the functions of hetero-oligomerization and D2-interacting proteins should lead to development of novel therapeutics for neuropsychiatric disorders related to dopamine system dysfunction.

Calcium Signaling of Hetero-Oligomeric Complexes Containing the D2 Receptor

Interestingly, D1 (or D5) and D2 receptors form functional hetero-oligomeric complexes in vitro and in vivo [3]. The D1/D2 hetero-oligomer activates calcium signaling through a novel $G_{q/11}$ protein-linked phospholipase C [3]. Combined stimulation with D1 and D2 full agonists such as SKF38393 and quinpirole, respectively, causes a robust increase in intracellular Ca^{2+} concentration through $G_{q/11}$ signaling. The physiological relevance of D1/D2 receptor hetero-oligomers is documented in neurons found in several brain regions. In cortical neurons cultured from fetal rat cortex, most pyramidal-like neurons express both D1 and D2 receptors and form D1/D2 receptor complexes. Importantly, long-term incubation with high concentrations of dopamine (100 μM) or SKF83959 (50 μM), a D1/D2 hetero-oligomer receptor agonist, significantly induces neuronal apoptosis [12]. D1/D2 hetero-oligomer receptor-induced apoptosis is associated with calcium elevation and generation of reactive oxygen species, followed by caspase 3/7 activation and mitochondrial cytochrome C release, suggesting involvement of a dopamine-driven apoptotic pathway in neurodegenerative disorders. On the other hand, stimulation of cortical neurons with SKF38393 and quinpirole alone, which are D1 and D2 receptor agonists, respectively, has no apoptotic effect through D1/D2 hetero-oligomeric receptors [12]. Since prolonged incubation with high concentrations of dopamine is required to trigger apoptosis, D1/D2 hetero-oligomeric receptor-induced apoptosis is likely related to neuronal loss seen in schizophrenia and/or Parkinson's patients. In addition, overactivation of ERK signaling through D1/D3 hetero-oligomer by chronic L-DOPA treatment is known to be causative for L-DOPA-induced dyskinesis, a severe motor side effect, in which D3 receptor mediates an increased efficiency of D1 receptor coupling to adenylate cyclase in the striatum [13].

D1/D2 hetero-oligomeric receptor signaling in the striatum and nucleus accumbens is a crucial factor in drug abuse. In the nucleus accumbens and caudate putamen, D1/D2 hetero-oligomers are expressed in a subset of medium spiny neurons (MSNs) that express both dynorphin and enkephalin [14]. In these regions, D1/D2 hetero-oligomers are also expressed in the termini of corticostriatal glutamatergic neurons that innervate the cell body of MSNs. Fluorescence resonance energy transfer methods using fluorophore-tagged D1 and D2 receptors show significant functional relevance of D1/D2 hetero-oligomers in presynaptic nerve terminals in the nucleus accumbens and caudate putamen. In addition, increased numbers of striatal D1/D2 hetero-oligomer receptors are observed after amphetamine treatment, an activity likely related to amphetamine-induced behavioral sensitization [14].

The D2 hetero-oligomeric receptor with the adenosine A_{2A} receptor has also been extensively studied in the striatum [15]. In striatal neurons, A_{2A} and D2 hetero-oligomers are highly expressed in enkephalin-positive GABAergic MSNs [16]. Patch-clamp experiments demonstrate that D2 receptor stimulation suppresses *N*-methyl-D-aspartate (NMDA)-induced depolarized plateau potential in MSN neurons, and A_{2A} receptor stimulation reverses D2 receptor-mediated suppression. Notably, an inhibitory peptide (SAQEpS peptide) disrupting A_{2A} and D2 dimerization blocks A_{2A} receptor modulation, suggesting that these heterodimers function in vivo to modulate responses to glutamate in enkephalin-positive GABAergic MSNs [17]. These findings are of interest because the A_{2A} receptor has become an attractive target for development of therapeutics for Parkinson's disease [18].

As noted, Arg-rich domains of the N-terminal portion of the third cytoplasmic loop of D2 receptor are binding sites for both calmodulin (CaM) and the A_{2A} receptor C-terminus. CaM overexpression does not inhibit A_{2A}–D2 receptor heterodimerization but rather CaM binds to the C-terminus of the A_{2A} receptor in the A_{2A}–D2 dimer [19]. Such Ca^{2+}/CaM binding enhances D2 receptor agonist-induced ERK activation but inhibits A_{2A} receptor-induced ERK activation [15]. However, the pharmacological relevance of ERK activation through A_{2A}–D2 receptor heterodimers remains unclear.

Regulation of Dopamine D2 Receptor Function through its Third Cytoplasmic Loop: Role of CaM, NR2B, and CaMKII

In hetero-oligomeric receptors composed of D2 receptor, the third cytoplasmic loop of D2 receptor and proteins that interact with this region is critical to define intracellular signaling, such as through ERK or calcium/CaM-dependent signaling. We first focus on CaM, *N*-methyl-D-aspartate receptor 2B subunit (NR2B), and calcium/calmodulin-dependent protein kinase II (CaMKII). CaM acts as a cellular Ca^{2+} sensor to activate ion channels and enzymes that regulate the cell cycle, cytoskeletal organization, and development. Bofill-Cardona et al. report a CaM-binding motif located in the N-terminus of the third cytoplasmic loop of D2 receptor at residues I210 to V223 [20]. This motif exhibits consensus hydrophobic residues (Val, Ile) at I210, I215, and V223 [21]. Ca^{2+}/CaM binding to the receptor interferes with D2 receptor signaling by inhibiting receptor-mediated G protein activation. CaM does not perturb G protein recognition but impedes receptor-induced activation [20]. By contrast, CaM does not bind to the third cytoplasmic domain moiety of a glutathione *S*-transferase (GST) fusion with the D3 receptor (R210–P239) [22].

NMDA receptors comprise a family of ionotropic glutamate receptors playing a central role in synaptic plasticity and memory formation [23]. NMDA receptors form by assembly of the principal NR1 subunit with different modulatory NR2 subunits (NR2A-D). The carboxyl tail of NR2B selectively binds to D2 but not D3 receptors through an N-terminal 10 amino acid motif T225–A234 in the third cytoplasmic domain of D2 receptor [24]. Although the NR2B binding motif is adjacent to the CaM binding domain at I210–V223, it does not bind CaM, strongly suggesting that NR2B–D2 interactions do not affect CaM binding to the D2 receptor. However, NR2B–D2 receptor interactions disrupt NR2B–CaMKII interaction, inhibiting NR2B phosphorylation at S1303 by CaMKII [24]. Furthermore, NR2B–D2 interaction inhibits NMDA receptor-mediated currents in striatal medium-sized striatal neurons. Behaviorally, NR2B antagonists mimic the effects of D2 receptor agonists in enhancing cocaine-stimulated motor activity and stereotyped behavior [24].

CaMKII, a multifunctional protein kinase that regulates biosynthesis and exocytosis of neurotransmitters and synaptic plasticity, is highly expressed in the central nervous system, especially in the hippocampal formation [25]. CaMKII autophosphorylation converts it from a Ca^{2+}-dependent to Ca^{2+}-independent species. Elevation of intracellular Ca^{2+} levels in cultured neurons by NMDA receptor stimulation results in increased CaMKII autophosphorylation at Thr286 of the α subunit and Thr287 of the β subunit, with concomitant elevation of Ca^{2+}-independent activity. Increased CaMKII autophosphorylation is essential for long-lasting increases in synaptic efficacy seen following LTP in the hippocampus [26]. CaMKIIα directly binds to D3 receptor fragments containing a 30 amino acid region from residues R210 to P239 at the N-terminus of the third cytoplasmic loop in vitro [22]. The CaMKIIα interacting region binds to the CaMKIIα catalytic domain. Furthermore, CaMKIIα phosphorylates D3 receptor at Ser229 in the R210–P239 motif. In accumbal neurons in vivo, Ca^{2+} stimulates binding of CaMKII to D3 receptor and increases its phosphorylation, suppressing D3 receptor function [22]. In addition, the D1/D2 heteroreceptor agonist SKF 83959 activates CaMKIIα in vivo in the mouse striatum [27]. Although cocaine-induced increases in MSN spine density are differently regulated in distinct D1 or D2 receptor-expressing neurons in the nucleus accumbens [28], D1/D2 oligomers also regulate spine density in a CaMKII-dependent manner in the striatum [29].

Prostate apoptosis response-4 (Par-4) is a leucine zipper-containing protein initially identified as a pro-apoptotic factor [30]. In the nervous system, Par-4 induction is linked to neuronal cell death in neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis. Par-4 was shown to be a D2 receptor-interacting protein in a yeast two-hybrid screen of a human

fetal embryonic brain library using the third intracellular loop of human D2L receptor (I212–Q373) as bait [31]. Furthermore, in vitro binding assays identified the domain of D2 receptor interacting with Par-4 as the first 30 amino acid residues of the third intracellular loop (amino acid residues I212–K241). Since CaM binds to the D2 receptor in the same region (I210–V223), CaM likely competes for binding with Par-4 in a Ca^{2+}-dependent manner, which would result in reduced D2 receptor efficacy and relieve D2 receptor-mediated inhibition of cAMP signaling [31]. In addition, disruption of Par-4 and D2 receptor interaction in transgenic mice expressing a Par-4 mutant promoted an increase in depression-like behaviors in forced swim and tail suspension tests [31]. Par-4 expression levels in significantly depressed or schizophrenic patients are also significantly decreased compared to normal controls [32]. Taken together, Ca^{2+}/CaM competition with Par-4 on the D2 receptor is likely important in regulating the apoptotic effects of Par-4 on cells that express D2 receptor. These interactions between CaM and Par-4 may function in depressive and schizophrenia patients.

H-FABP is a Novel Protein That Interacts with the D2L Receptor Third Cytoplasmic Loop

As noted, proteins interacting with the D2 receptor third cytoplasmic loop have been identified using yeast two-hybrid systems and in vitro and in vivo binding assays. However, factors cited above interact with regions common to the third cytoplasmic loop of D2L and D2S receptors: the 29 amino acid insert (G242–V270) found only in D2L receptor does not participate in those interactions. Although functional differences between two isoforms remain unclear, a specific interaction of the D2L receptor with $G\alpha_{i2}$ [33] and the differential subcellular localization of D2 receptor isoforms are evident [34]. The D2S receptor is mainly localized at the plasma membrane, whereas the D2L receptor is retained primarily around the endoplasmic reticulum and Golgi apparatus when expressed in COS-7 and HeLa cells [34]. In neuroblastoma NG108-15 cells, we confirmed different subcellular localization of two D2 receptor isoforms [35]. Significant Ca^{2+}-dependent signaling was observed in NG108-15 cells stably expressing D2L receptor following transfection as compared to D2S receptor [36]. Ca^{2+}-dependent signaling in D2L receptor-transfected cells is associated with nuclear CaMKII activation, thereby increasing CaMKII-dependent brain-derived neurotrophic factor (BDNF) expression in NG108-145 cells, suggesting that D2L/$G\alpha_{i2}$-coupled signaling likely stimulates nuclear Ca^{2+} signaling in neurons. Overexpression of nuclear CaMKIIδ 3 potentiates the BDNF gene transcription containing exon 4 in NG108-15 cells [36]. Since stimulation of the D1/D2 hetero-oligomeric receptor causes robust Ca^{2+} mobilization, CaMKII likely functions in BDNF expression in the nucleus accumbens.

Using the yeast two-hybrid system, we have identified for the first time heart-type fatty acid binding protein (H-FABP, also named as FABP3) as binding to the 29 amino acid domain found only in the D2L receptor [35]. When D2L or D2S receptor is co-expressed with H-FABP in NG108-15 cells, overexpressed and endogenous H-FABP clearly co-localizes with only the D2L receptor in the Golgi apparatus and endoplasmic reticulum but not in the plasma membrane. In immunoprecipitation assays of transfected NG108-15 cells, we confirmed interaction between H-FABP with D2L receptor-YFP but not with D2S receptor-YFP. Brain-type FABP (B-FABP) did not bind to D2L receptor-YFP [35]. Likewise, in striatal cell extracts, immunocomplexes with the anti-D2L receptor antibody included H-FABP in wild-type mice but did not in an H-FABP knockout (KO) mice [37].

The striatal microcircuit is composed of MSNs that receive excitatory corticostriatal glutamatergic innervation, dopaminergic nigrostriatal fibers, and cholinergic interneuron terminals. Strong H-FABP immunoreactivity is detected in all cholinergic neurons in the mouse striatum (Fig. 1). H-FABP protein is also expressed in glutamatergic terminals in the dorsal striatum, whereas H-FABP is not present in dopaminergic terminals or in postsynaptic densities in dendritic spines of MSNs in the dorsal striatum. In H-FABP-expressing regions, D2 receptor is also present in cell bodies of cholinergic neurons and in glutamatergic terminals in the dorsal striatum [37]. Interestingly, H-FABP KO mice exhibit D2 receptor dysfunction, based on evaluation of dopamine-related behaviors [37]. Specifically, H-FABP KO mice show reduced responsiveness to methamphetamine (METH)-induced sensitization of locomotor activity compared to wild-type mice (Fig. 2). Enhanced haloperidol-induced catalepsy is also

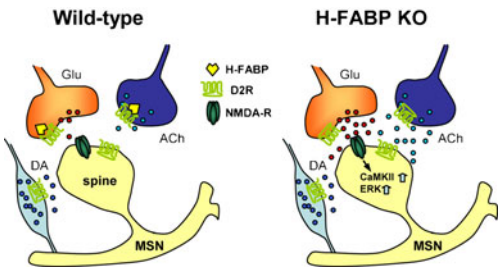

Fig. 1 Working hypothesis of altered neurotransmission in the dorsal striatum following H-FABP deletion. *Left*, the striatal microcircuit is composed of medium spiny neurons (MSNs) that receive input from excitatory corticostriatal glutamatergic projections, dopaminergic nigrostriatal fibers, and cholinergic terminals. D2R is present at the postsynapse (spine) of MSNs and in each terminal. However, H-FABP is present only in glutamatergic and cholinergic terminals. H-FABP binds to the third cytoplasmic loop of D2R. *Right*, following H-FABP deletion, D2R inhibitory effects are disrupted, resulting in increased Glu and ACh release from respective glutamatergic and cholinergic terminals and CaMKII and ERK activation at MSN postsynapses (from [37])

observed in H-FABP KO compared to wild-type mice (Fig. 2). Notably, catalepsy induced by the D1 antagonist SCH23390 is not altered by the lack of H-FABP, suggesting that only D2 receptor function is impaired in the absence of H-FABP. Consistent with aberrant potentiation of haloperidol-induced catalepsy, haloperidol-induced acetylcholine (ACh) release is markedly increased in the dorsal striatum of H-FABP KO mice without changes in haloperidol-induced glutamate (Glu) release (Fig. 3). However, depolarization-induced Glu release is enhanced in the dorsal striatum of H-FABP KO mice. Indeed, CaMKII and ERK activities are aberrantly increased in the dorsal striatum in the absence of drug stimulation, suggesting that basal Glu release from corticostriatal excitatory neurons innervating to striatal neurons is enhanced in H-FABP KO mice. In the striatum, haloperidol stimulates ERK phosphorylation in D2 receptor-expressing MSNs but not in D1-expressing MSNs [38]. Like H-FABP KO mice, D2 receptor KO mice show markedly reduced cocaine-induced behavioral sensitization [39] and increases in basal Glu release in the corticostriatal pathway [40]. Taken together, H-FABP expressed in ACh interneurons and terminals of glutamatergic neurons likely regulates ACh and Glu release in the mouse dorsal striatum through interaction with the D2L receptor

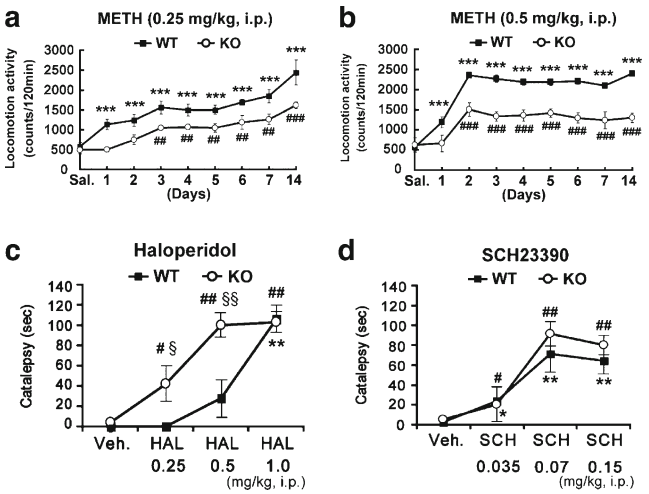

Fig. 2 Reduced behavioral responses to methamphetamine and haloperidol in H-FABP KO mice. **a**, **b** Behavioral sensitization to methamphetamine (*METH*) (**a** 0.25 mg/kg or **b** 0.5 mg/kg) in H-FABP KO mice was significantly reduced compared with wild-type mice. Each data point represents the mean ± SEM. ***$p<0.001$, versus saline-treated (day 0) wild-type mice; $^{\#\#}p<0.01$ and $^{\#\#\#}p<0.001$, versus saline-treated (day 0) H-FABP KO mice. **c** H-FABP KO mice exhibit strong haloperidol-induced catalepsy compared with wild-type mice. Each data point represents the mean ± SEM. **$p<0.01$, versus vehicle-treated (*Veh.*) wild-type mice; $^{\#}p<0.05$, $^{\#\#}p<0.01$, versus vehicle-treated (*Veh.*) H-FABP KO mice. $^{\S}p<0.05$, $^{\S\S}p<0.01$ in H-FABP KO versus wild-type mice at the same dosage. **d** SCH23390-induced catalepsy did not differ significantly between wild-type and H-FABP KO mice. Each data point represents the mean ± SEM. *$p<0.05$ and **$p<0.01$ versus vehicle-treated (*Veh.*) wild-type mice; $^{\#}p<0.05$ and $^{\#\#}p<0.01$ versus vehicle-treated (*Veh.*) H-FABP KO mice. *WT* wild-type mice, *KO* H-FABP KO mice (from [37])

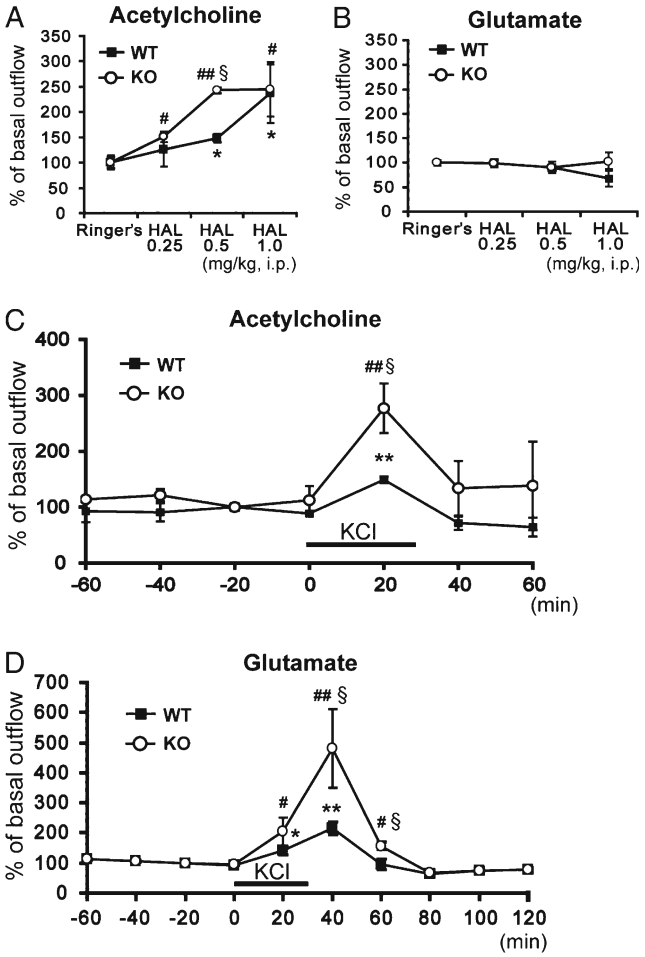

Fig. 3 Enhanced ACh and Glu release in the dorsal striatum seen in freely moving H-FABP KO compared to wild-type mice. **a** Extracellular ACh changes following intraperitoneal haloperidol (*HAL*) administration. H-FABP KO mice showed markedly increased haloperidol-induced ACh release compared with wild-type mice at a dosage of 0.5 mg/kg. $^{\S}p<0.05$, in H-FABP KO mice versus wild-type mice at the same dose. *$p<0.05$, versus basal levels in wild-type mice; $^{\#}p<0.05$ and $^{\#\#}p<0.01$, versus basal levels in H-FABP KO mice. **b** Extracellular Glu changes following intraperitoneal haloperidol administration. **c** KCl depolarization-induced ACh release in the dorsal striatum. Each data point represents the mean ± SEM. $^{\S}p<0.05$, H-FABP KO mice versus wild-type mice at the same dosage. **$p<0.01$, versus basal levels in wild-type mice; $^{\#\#}p<0.01$, versus basal levels in H-FABP KO mice. **d** KCl depolarization-induced Glu release in the dorsal striatum. Each data point represents the mean ± SEM. $^{\S}p<0.05$, in H-FABP KO versus wild-type mice at the same dosage. *$p<0.05$ and **$p<0.01$, versus basal levels in wild-type mice; $^{\#}p<0.05$ and $^{\#\#}p<0.01$, versus basal levels in H-FABP KO mice. *WT* wild-type mice, *KO* H-FABP KO mice (from [37])

(Fig. 1). ACh release from interneurons is mainly involved in haloperidol-induced catalepsy, whereas Glu release from corticostriatal termini is not involved in haloperidol-induced catalepsy behavior in the dorsal striatum. Loss of H-FABP in ACh interneurons and glutamatergic terminals impairs D2 receptor regulation of ACh and Glu release in these regions. Overexpression of H-FABP in D2 receptor-expressing NG108-15 cells enhances ERK activity stimulated by

quinpirole, suggesting that enhanced striatal ERK activity in H-FABP KO mice does not directly account for enhanced catalepsy.

Notably, we have no clear evidences how the extracellular dopamine gain access to D2L receptor protein expressed in Golgi apparatus and/or endoplasmic reticulum, where D2L receptor should be coupled with G-proteins. There are several possibilities to activate intracellular signaling such as ERKs. Dopamine D4 receptor transactivated intracellular platelet-derived growth factor receptor (βPDGFRβ), thereby stimulating ERKs [41]. The intracellular less maturely glycosylated PDGFRβ is sufficient to facilitate D4 receptor-mediated ERK activation [41]. Similarly, cell surface deficient mutants of the V2 vasopressin receptor are intracellularly activated during its biosynthesis by a new class of non-peptide plasma membrane-permeable agonists [42, 43]. We also notice that immunoelectron microscopic studies of Golgi preparations show that both Gai-2 and Gai-3 are localized at multivesicular structure identified as Golgi complexes [44]. Taken together, internalized dopamine D2 receptor upon prolonged stimulation or cell permeable agonist including hydrophobic agonists such as quinpirole likely activates intracellular pooled D2L receptor associated with Golgi apparatus. Further extensive studies are required to define the physiological relevance of G-protein-coupled signaling complexes in the Golgi apparatus.

NSF, DAT, and Spinophilin Interact with the C-Terminal Region of the Third Cytoplasmic Loop of Dopamine D2 Receptors

N-ethylmaleimide-sensitive factor (NSF) is a homohexameric ATPase [45] that is an essential component of protein machinery responsible for various membrane fusion events, including intercisternal Golgi protein transport and exocytosis of synaptic vesicles. Direct interaction between the carboxyl tail of the AMPA receptor GluR2 subunit and NSF has been documented [46]. Studies employing co-immunoprecipitation, affinity purification, and in vitro binding assays also show that NSF directly binds to the third cytoplasmic loop of D2 receptor at F341-Q373 [47]. D2 receptor activation increases this interaction, resulting in decreased D2 receptor–GluR2 interaction. This result suggests that NSF regulates interaction between the two receptors, depending on the activation state of the D2 receptor [47].

The dopamine transporter (DAT) is a membrane-bound protein that facilitates reuptake of extracellular DA and is a target for drugs of abuse, including cocaine and amphetamine. DAT is a major presynaptic protein involved in regulating dopaminergic tone. Lee et al. provide evidence for direct interaction between the third cytoplasmic loop of D2R (I340-Q373) and DAT [48]. Utilizing transfected cells and GST fusion proteins, they employed the D2SR isoform in experiments, as DAT interacts with a region common to both D2SR and D2LR. DAT recruitment to the plasma membrane, which is essential for its function, is promoted by D2–DAT interaction. Since mice injected with peptides disrupting the DAT/D2R association exhibit decreased synaptosomal dopamine uptake and increased locomotor activity, interaction between these proteins may contribute to dopaminergic tone and activity within the brain. The third cytoplasmic loop of D2 receptor binds to spinophilin, a protein phosphatase-1 (PP-1)-binding protein that interacts in the postsynaptic density with AMPA receptors through its PDZ domain [49]. Spinophilin regulates spine density and morphology though F-actin binding [50]. Indeed, dopaminergic termini innervate the corticostriatal excitatory pathways and modulate spine morphology through the D2 receptor/spinophilin/protein phosphatase-1 complex [51].

Novel D2 Receptor-Dependent Akt Signaling through β-Arrestin 2 Interaction

β-Arrestin 1 and β-arrestin 2 function in desensitization of G-protein-coupled receptors (GPCRs) after phosphorylation by GPCR kinases (GRKs). Since β-arrestin 1 or β-arrestin 2 KO mice show reduced responsiveness to dopamine receptor agonists, β-arrestins likely play a critical role in psychomotor behaviors through regulation of receptor internalization in a GRK-dependent manner. β-Arrestin 1 KO mice show reduced responsiveness to cocaine [52], whereas β-arrestin 2 KO mice exhibit blunted locomotor activity in response to amphetamine and morphine [53]. β-Arrestin 2 interacts with the DRY motif in the second cytoplasmic loop of dopamine receptors [54]. Upon phosphorylation of the C-terminal tail of GPCRs, β-arrestin binds GPCR and recruits dynamin and clathrin to activate the endocytotic pathway. The internalized GPCR/β-arrestin complex also triggers ERK activation. Moreover, prolonged D2 receptor stimulation leads to dephosphorylation of Akt (Thr308), decreasing its activity. Amphetamine-induced D2 receptor activation promotes complex formation between Akt and PP2A through β-arrestin 2 as a scaffold protein, triggering dephosphorylation of Akt (Thr308), which is required for Akt activity. Reduced Akt activity leads to activation of glycogen synthase kinase-3β, which is significantly involved in neurological and psychiatric disorders, such as Alzheimer's disease and schizophrenia [55].

Fabps Are Targets for Long Chain Polyunsaturated Fatty Acids

Schizophrenic patients exhibit significantly lower levels of long chain polyunsaturated fatty acids (LCPUFAs),

including arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in red blood cells [56]. Several clinical studies indicate that oral administration of EPA can improve emotional and cognitive function in these patients [57]. Likewise, AA [58] and DHA [59] supplementation can improve cognitive dysfunction seen in disorders such as Alzheimer's disease and schizophrenia. Since LCPUFAs are insoluble in an aqueous environment, FABPs are essential to function as cellular shuttles to transport LCPUFAs to appropriate intracellular compartments. Small 14–15-kDa cytoplasmic FABPs belong to a family consisting of at least 13 different widely distributed proteins. Among various FABPs, brain- (B-), epidermal- (E-), and heart- (H-) type FABPs are all expressed in the brain [60]. Indeed, lack of H-FABP reduces AA content in phosphatidyl inositol in brain phospholipid fractions [61]. B-FABP knockdown by small interfering RNA in cortical neuroepithelial cells impairs cell proliferation and promotes neuronal differentiation [62]. B-FABP KO mice show abnormalities in emotional behavior, decreased neurogenesis in the dentate gyrus, and impaired prepulse inhibition [63]. Given phenotypes exhibited by H-FABP KO mice in our study, the crucial role of H-FABP in D2L receptor signaling may explain the clinical relevance of LCPUFAs in ameliorating emotional and cognitive behaviors seen in schizophrenia and Alzheimer's diseases. Since H-FABP is highly expressed in a subset of neurons in the medial prefrontal cortex and hippocampus (unpublished observations), our goal should be to define phospholipid composition/metabolism and dopamine D2 receptor signaling in H-FABP-positive neurons to further understand the physiological relevance of H-FABP in D2 receptor function.

Conclusion

Notably, the activities of dopamine D2 receptor are mediated not only by heterotrimeric G proteins but also by proteins interacting specifically with the third cytoplasmic loop. Interaction with NR2B, calmodulin, CaMKII, Par-4, DAT, NSF, and spinophilin likely mediates synaptic plasticity related to memory and emotional behaviors. These interacting proteins also modulate function of hetero-oligomeric receptors with other GPCRs. Ca^{2+}-dependent signaling through D1/D2 and D2/A_{2A} hetero-oligomeric receptors is particularly important for drug abuse and psychomotor behaviors. Prolonged stimulation of the Ca^{2+}-mobilizing receptors is likely implicated in neurodegenerative disorders including Parkinson's disease and schizophrenia. We also emphasize the physiological role of H-FABP interacting with the D2L receptor third cytoplasmic loop. Analysis of D2L receptor dysregulation in H-FABP KO mice should further promote our understanding of D2L receptor in regulation of extrapyramidal symptoms through the nigrostriatal dopamine system.

References

1. Missale C, Nash R, Robinson SW, Jaber M, Caron MG (1998) Dopamine receptors from structure to function. Physiol Rev 78:189–225
2. Sidhu A, Niznik HB (2000) Coupling of dopamine receptor subtypes to multiple and diverse G proteins. Int J Dev Neurosci 18:669–677
3. Rashid AJ, So CH, Kong MM, Furtak T, El-Ghundi M, Cheng R, O'Dowd BF, George SR (2007) D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. Proc Natl Acad Sci USA 104:654–659
4. So CH, Verma V, Alijaniaram M, Cheng R, Rashid AJ, O'Dowd BF, George SR (2009) Calcium signaling by dopamine D5 receptor and D5-D2 receptor hetero-oligomers occurs by a mechanism distinct from that for dopamine D1-D2 receptor hetero-oligomers. Mol Pharmacol 75:843–854
5. Oak JN, Lavine L, Van Tol HHM (2001) Dopamine D4 and D2L receptor stimulation of the mitogen-activated protein kinase pathway id dependent on trans-activation of the platelet-derived growth factor receptor. Mol Pharmacol 60:92–103
6. Dal Toso R, Sommer B, Ewert M, Herb A, Pritchett DB, Bach A, Shivers BD, Seeburg PH (1989) The dopamine D_2 receptor: two molecular forms generated by alternative splicing. EMBO J 8:4025–4034
7. Itokawa M, Arinami T, Futamura N, Hamaguchi H, Toru M (1993) A structural polymorphism of human dopamine D2 receptor, D2 (Ser311→Cys). Biochem Biophys Res Commun 196:1369–1375
8. Arinami T, Itokawa M, Enguchi H, Tagaya H, Yano S, Shimizu H, Hamaguchi H, Toru M (1994) Association of dopamine D2 receptor molecular variant with schizophrenia. Lancet 343:703–704
9. Laurent C, Bodeau-Pean S, Campion D et al (1994) No major role for the dopamine D2 receptor Ser→Cys311 mutation in schizophrenia. Psychiatr Genet 4:229–230
10. Cravchik A, Sibley DR, Gejman PV (1996) Functional analysis of the human D_2 dopamine receptor missense variants. J Biol Chem 271:26013–26017
11. Zhang Y, Bertolino A, Fazio L, Blasi G, Rampino A, Romano R et al (2007) Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. Proc Natl Acad Sci USA 104:20552–20557
12. Zhang L, Yang H, Zhao H, Zhao C (2011) Calcium-related signaling pathways contributes to dopamine-induced cortical neuron apoptosis. Neurochem Internat 58:281–294
13. Fiorentini C, Busi C, Spano P, Missale C (2010) Dimerization of dopamine D1 and D3 receptors in the regulation of striatal function. Curr Opin Phamacol 10:87–92
14. Perreault ML, Hasbi A, Alijaniaram M, Fan T, Varghese G, Fletcher PJ, Seeman P, O'Dowd BF, George SR (2010) The dopamine D1–D2 receptor heteromer localizes in dynorphin/encephalin neurons. J Biol Chem 285:36625–36634
15. Ferré S, Woods AS, Navarro G, Aymerich M, Lluis C, Franco R (2010) Calcium-mediated modulation of the quaternary structure and function of adenosine A2A-dopamine D2 receptor heteromers. Curr Opin Pharmacol 10:67–72
16. Schiffmann SN, Fisone G, Moresco R, Cunha RA, Ferré S (2007) Adenosine A2A receptors and basal ganglia physiology. Prog Neurobiol 83:277–292
17. Azdad K, Gall D, Woods AS, Ledent C, Ferré S, Schiffmann SN (2009) Dopamine D2 and A2A receptors regulate NMDA-

mediated excitation in accumbens neurons thorugh A2A-D2 receptor heteromerization. Neuropsychopharmacol 34:972–986
18. Xu K, Bastia E, Schwarzschild M (2005) Therapeutic potential of adenosin2 A2A receptor antagonists in Parkinson's disease. Pharmacol & Therapeutics 105:267–310
19. Navarro G, Aymeric MS, Marcellino D, Cortes A, Casado V, Mallol J, Canela EI, Agnati LF, Woods AS, Fuxe K et al (2009) Interactions between calmodulin, adenosine A_{2A} and dopamine D2 receptors. J Biol Chem 284:28058–28068
20. Bofill-Cardona E, Kudlacek O, Yang Q, Ahorn H, Freissmuth M, Nanoff C (2000) Binding of calmodulin to the D2-dopamine receptor reduces receptor signaling by arresting the G protein activation switch. J Biol Chem 275:32672–32680
21. Rhoads AR, Friedberg F (1997) Sequence motifs for calmodulin recognition. FASEB J 11:331–340
22. Liu XY, Mao LM, Zhang GC, Papasian CJ, Fibuch EE, Lan HX, Zhou HF, Xu M, Wang JQ (2009) Activity-dependent modulation of limbic dopamine D3 receptors by CaMKII. Neuron 61:425–438
23. Collingridge GL, Singer W (1990) Excitatory amino acid receptors and synaptic plasticity. Trends Pharmacol Sci 11:290–296
24. Liu XY, Chu XP, Mao LM, Wang M, Lan HX, Li MH, Zhang GC, Parelkar NK, Fibuch EE, Haines M, Neve KA, Liu F, Xiong ZG, Wang JQ (2006) Modulation of D2R–NR2B interactions in response to cocaine. Neuron 52:897–909
25. Ouimet CC, McGuinness TL, Greengard P (1984) Immunocytochemical localization of calcium/calmodulin-dependent protein kinase II in rat brain. Proc Natl Acad Sci USA 81:5604–5608
26. Fukunaga K, Stoppini L, Miyamoto E, Muller D (1993) Long-term potentiation is associated with an increased activity of Ca^{2+}/calmodulin-dependent protein kinase II. J Biol Chem 268:7863–7867
27. Ng J, Rashi AJ, So CH, O'Dowd BF, George SR (2010) Activation of CaMKIIα in the striatum by the heteromeric D1/D2 dopamine receptor complex. Neuroscience 165:535–541
28. Lee K-W, Kim Y, Kim AM, Helmin K, Nairn AC, Greengard P (2006) Cocaine-induced dendric spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. Proc Natl Acad Sci USA 103:3399–3404
29. Shioda N, Beppu H, Fukuda T, Li E, Kitajima I, Fukunaga K (2011) Aberrant calcium/calmodulin-dependent protein kinase II (CaMKII) activity is associated with abnormal dendritic spine morphology in the ATRX mutant mouse brain. J Neurosci 31: 346–358
30. Sells SF, Wood DP Jr, Joshi-Barve SS, Muthukumar S, Jacob RJ, Crist SA, Humphreys S, Rangnekar VM (1994) Commonality of the gene programs induced by effectors of apoptosis in androgen-dependent and -independent prostate cells. Cell Growth Differ 5:457–466
31. Park SK, Nguyen MD, Fischer A, Luke MP, Affar el B, Dieffenbach PB, Tseng HC, Shi Y, Tsai LH (2005) Par-4 links dopamine signaling and depression. Cell 122:275–287
32. Glantz LA, Gilmore JH, Overstreet DH, Salimi K, Lieberman JA, Jarkog LF (2010) Pro-apoptotic Par-4 and dopamine D2 receptor in temporal cortex in schizophrenia, bipolar disorder and major depression. Schizophrenia Res 118:292–299
33. Guiramand J, Montmayeur JP, Ceraline J, Bhatia M, Borrelli E (1995) Alternative splicing of the dopamine D2 receptor directs specificity of coupling to G-proteins. J Biol Chem 270:7354–7358
34. Prou D, Gu WJ, Le S, Vincent JD, Salamero J, Vernier P (2001) Intracellular retention of the two isoforms of the $D_{(2)}$ dopamine receptor promotes endoplasmic reticulum disruption. J Cell Sci 114:3517–3527
35. Takeuchi Y, Fukunaga K (2003) Differential subcellular localization of two dopamine D2 receptor isoforms in transfected NG108-15 cells. J Neurochem 85:1064–1074
36. Takeuchi Y, Fukunaga K, Miyamoto E (2002) Activation of nuclear Ca^{2+}/calmodulin-dependent protein kinase II and brain-derived neutropic factor gene expression by stimulation of dopamine D2 receptor in transfected NG108-15 cells. J Neurochem 82: 316–328
37. Shioda N, Yamamoto Y, Watanabe M, Binas B, Owada Y, Fukunaga K (2010) Heart-type fatty acid binding protein regulates dopamine D2 receptor function in mouse brain. J Neurosci 30: 3146–3155
38. Bertran-Gonzalez J, Bosch C, Maroteaux M, Matamales M, Hervé D, Valjent E, Girault JA (2008) Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. J Neurosci 28: 5671–5685
39. Welter M, Vallone D, Samad TA, Meziane H, Usiello A, Borrelli E (2007) Absence of dopamine D2 receptors unmasks an inhibitory control over the brain circuitries activated by cocaine. Proc Natl Acad Sci USA 104:6840–6845
40. Cepeda C, Hurst RS, Altemus KL, Flores-Hernández J, Calvert CR, Jokel ES, Grandy DK, Low MJ, Rubinstein M, Ariano MA, Levine MS (2001) Facilitated glutamatergic transmission in the striatum of D2 receptor-deficient mice. J Neurophysiol 85:659–670
41. Gill RS, Hsiung MS, Sum CS, Lavine N, Clark SD, Van Tol HHM (2010) The dopamine D4 receptor activates intracellular platelet-derived growth factor receptor b to stimulate ERK1/2. Cell Signaling 22:285–290
42. Robben JH, Kortenoeven ML, Sze M, Yae C, Milligan G, Oorschot VM, Klumperman J, Knoers NV, Deen PM (2009) Intracellular activation of vasopressin V2 receptor mutants in nephrogenic diabetes insipidus by nonpeptide agonists. Proc Natl Acad Sci U S A 106:12195–12200
43. Jean-Alphonse F, Hanyaloglu AC (2011) Regulation of GPCR signal networks via membrane trafficking. Mol Cell Endcrinol 331:205–214
44. Maier O, Ehmsen E, Westermann P (1995) Trimetric G protein α subunits of the Gs and Gi families localized at the Golgi membrane. Biochem Biophy Res Commun 208:135–143
45. Hanson PI, Otto H, Barton N, Jahn R (1995) The N-ethylmaleimide-sensitive fusion protein and alpha-SNAP induce a conformational change in syntaxin. J Biol Chem 270:16955–16961
46. Nishimune A, Isaac JT, Molnar E, Noel J, Nash SR, Tagaya M, Collingridge GL, Nakanishi S, Henley JM (1998) NSF binding to GluR2 regulates synaptic transmission. Neuron 21: 87–97
47. Zou S, Li L, Pei L, Vukusic B, Van Tol HH, Lee FJ, Wan Q, Liu F (2005) Protein–protein coupling/uncoupling enables dopamine D2 receptor regulation of AMPA receptor-mediated excitotoxicity. J Neurosci 25:4385–4395
48. Lee FJ, Pei L, Moszczynska A, Vukusic B, Fletcher PJ, Liu F (2007) Dopamine transporter cell surface localization facilitated by a direct interaction with the dopamine D2 receptor. EMBO J 26: 2127–2136
49. Smith FD, Oxford GS, Milgram SL (1999) Association of the D2 dopamine receptor third cytoplasmic loop with spinophilin, a protein phosphatase-1-interacting protein. J Biol Chem 274:19894–19900
50. Feng J, Yan Z, Ferreira A, Tomizawa K, Liauw JA, Zhou M, Allen PB, Ouimat CC, Greenfard P (2000) Spinophilin regulates the formation and function od dendritic spines. Proc Natl Acad Sci USA 97:9287–9292
51. Yao WD, Spealman RD, Zhang J (2008) Dopaminergic signaling in dendritic spines. Biochem Pharmacol 75:2055–2069
52. Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG (2004) Desensitization of G protein-coupled receptors and neuronal functions. Annu Rev Neurosci 27:107–144
53. Beaulieu JM, Sotnikova TD, Merion S, Lefkowitz RJ, Gainetdinov RR, Caron MG (2005) An Aktβ-arrestin 2/PP2A signaling

complex mediates dopaminergic neurotransmission and behavior. Cell 122:261–273
54. Kim K-M, Caron MG (2008) Complementary roles of the DRY motif and C-terminus tail of GPCRs for G protein coupling and β-arrestin interaction. Biochem Biophy Res Commun 366:42–47
55. Li Y-C, Gao W-J (2011) GSK-3β activity and hyperdopamine-dependent behaviors. Neurosci Biobehav Reviews 35:645–654
56. Peet M, Laugharne J, Rangarajan N, Horrobin D, Reynolds G (1995) Depleted red cell membrane essential fatty acids in drug-treated schizophrenic patients. J Psychiatr Res 29:227–232
57. Arvindakshan M, Ghate M, Ranjekar PK, Evans DR, Mahadik SP (2003) Supplementation with a combination of omega-3 fatty acids and antioxidants (vitamins E and C) improves the outcome of schizophrenia. Schizophr Res 62:195–204
58. Kotani S, Sakaguchi E, Warashina S, Matsukawa N, Ishikura Y, Kiso Y, Sakakibara M, Yoshimoto T, Guo J, Yamashima T (2006) Dietary supplementation of arachidonic and docosahexaenoic acids improves cognitive dysfunction. Neurosci Res 56:159–164
59. Horrocks LA, Yeo YK (1999) Health benefits of docosahexaenoic acid (DHA). Pharmacol Res 40:211–225
60. Owada Y, Yoshimoto T, Kondo H (1996) Spatio-temporally differential expression of genes for three members of fatty acid-binding proteins in developing and mature rat brains. J Chem Neuroanat 12:113–122
61. Murphy EJ, Owada Y, Kitanaka N, Kondo H, Glatz JFC (2005) Brain arachidonic acid incorporation is decreased in heart fatty acid binding protein gene-ablated mice. Biochemistry 44:6350–6360
62. Arai Y, Funatsu N, Numayama-Tsuruta K, Nomura T, Nakamura S, Osumi N (2005) Role of Fabp7, a downstream gene of Pax6, in the maintenance of neuroepithelial cells during early embryonic development of the rat cortex. J Neurosci 25:9752–9761
63. Watanabe A, Toyota T, Owada Y, Hayashi T, Iwayama Y, Matsumata M, Ishitsuka Y, Nakaya A, Maekawa M, Ohnishi T, Arai R, Sakurai K, Yamada K, Kondo H, Hashimoto K, Osumi N, Yoshikawa T (2007) Fabp7 maps to a quantitative trait locus for a schizophrenia endophenotype. PLoS Biol 5:e297