学位論文

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Research article

MDGA1-deficiency attenuates prepulse inhibition with alterations of dopamine and serotonin metabolism: An *ex vivo* HPLC-ECD analysis

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ABSTRACT

MDGA1 (MAM domain-containing glycosylphosphatidylinositol anchor) has recently been linked to schizophrenia and bipolar disorder. Dysregulation of dopamine (DA) and serotonin (5-HT) systems has long been associated with schizophrenia and other neuropsychiatric disorders. Here, we measured prepulse inhibition (PPI) of the startle response and *ex vivo* tissue content of monoamines and their metabolites in the frontal cortex, striatum and hippocampus of *Mdga1* homozygous (*Mdga1*-KO), *Mdga1* heterozygous (*Mdga1*-HT) and wild-type (WT) male mice. We found that *Mdga1*-KO mice exhibited statistically significant impairment of PPI, and had higher levels of homovanillic acid in all three brain regions studied compared with *Mdga1*-HT and WT mice ($P < 0.05$), while levels of norepinephrine, DA and its metabolites 3,4-dihydroxyphenylacetic acid and 3-methoxytyramine remained unchanged. *Mdga1*-KO mice also had a lower 5-hydroxyindoleacetic acid level in the striatum ($P < 0.05$) compared with WT mice. 5-HT levels remained unchanged with the exception of a significant increase in the level in the cortex. These data are the first evidence suggesting that MDGA1 deficiency leads to a pronounced deficit in PPI and plays an important role in perturbation of DA and 5-HT metabolism in mouse brain; such changes may contribute to a range of neuropsychiatric disorders.

1. Introduction

Dopamine (DA) and serotonin (5-HT) are major neurotransmitters involved in several neurological functions in both the central nervous system (CNS) and the periphery [1–3]. DA and 5-HT signaling are terminated by uptake or enzymatic breakdown. Monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT) play a major role in DA catabolism, with homovanillic acid (HVA) as the main end-product of DA. MAO plays a major role in 5-HT catabolism, with 5-hydroxyindole acetic acid (5-HIAA) as the main end-product of 5-HT. Dysfunctions in central monoaminergic systems are involved in the pathogenesis of many neurological and psychiatric disorders, including schizophrenia, depression, attention deficit hyperactivity disorder (ADHD) and Parkinson's disease [4–7]. Therefore, there have been many studies of monoamine metabolite levels in human cerebrospinal fluid (CSF) [8], human brains [9] and rodent brains [10,11]. The level of 5-HIAA, a major metabolite of 5-HT, is decreased in depressed individuals [12], and there is a strong association between low CSF 5-HIAA level and suicidal tendency [13]. 5-HIAA is also associated with bipolar disorder and ADHD, as is the DA metabolite HVA [14–17]. Determination of levels of monoamines and their metabolites in brain may provide information about the turnover rates of such amines, aiding discovery of novel drugs to treat monoamine-related disorders.

MDGA1 (MAM domain-containing glycosylphosphatidylinositol anchor) is a cell-surface glycoprotein [18,19]. Expression of this protein is highest in developing brain, but it is found throughout the adult brain [19–21]. MDGA1 is differentially expressed by different populations of neurons [22], and it plays roles in cell adhesion, migration, axon

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guidance and, in the developing brain, neuronal migration [18,23–25]. Nevertheless, there is little apparent change in the gross anatomy of *Mdga1*-knockout (*Mdga1*-KO) mouse brains [25], suggesting that the protein plays a role in later neurodevelopmental stages. MDGA1 is important for the balanced operation of excitatory and inhibitory synapses. It binds to neuroligin 2, interfering with the ability of that protein to form a trans-synaptic bridge with neurexins, which decreases the ability of neuroligin 2 to promote inhibitory synapse formation [26,27]. There is a significant genetic association between MDGA1 and bipolar disorder and schizophrenia [28,29]. Schizophrenia is one of the most prominent psychiatric diseases, presenting with deficits in prepulse inhibition (PPI) of the startle response; the PPI decrease in schizophrenic patients [30,31] is thought to reflect an alteration in dopaminergic, serotonergic and glutamatergic neurotransmitter systems [32]. Accordingly, *Mdga1*-KO animals are an attractive model in which to measure PPI and the metabolism of monoamines in a disease-prone genetic background. However, no study has investigated the relationship between MDGA1-deficiency, PPI and monoamine metabolism in mouse brain.

Therefore, we chose *Mdga1*-KO, *Mdga1*-heterozygous (*Mdga1*-HT) and wild-type (WT) mice to measure *ex vivo* levels of NE, DA and 5-HT, and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HIAA, HVA and 3-methoxytyramine (3-MT) in the frontal cortex, striatum and hippocampus. The cortical, striatal and hippocampal behavioral systems are thought to operate independently or in parallel [33,34]. High-performance liquid chromatography with an electrochemical detector (HPLC-ECD) was used for simultaneous determination of monoamines and their metabolites. We conducted measurements of acoustic startle responses in mice using an LE 118–4 startle and fear interface.

2. Materials and methods

2.1. Animals

Mdga1-KO and *Mdga1*-HT and their wild-type (WT) littermates were bred and genotyped in Kagawa University Animal Building. *Mdga1*-KO mice were backcrossed with the C57BL/6 strain for more than 10 generations [25]. WT mice have the same genetic background as C57BL/6 mice and were bred in Kagawa University animal facility. Mice were housed in controlled temperature (21 ± 3 °C), humidity (50–70 %) and light (12-h light–dark cycle) conditions. All animal experiments were approved by the Kagawa University Animal Investigation Committee. Adult (10–11-weeks-old, 23–26 g body weight) *Mdga1*-KO, *Mdga1*-HT and WT mice (n = 7 for each) were used for the determination of monoamines and their metabolites. Adult *Mdga1*-KO (n = 8), *Mdga1*-HT (n = 8) and WT (n = 9) mice (12–16-weeks-old) were used for behavioral studies. All experiments were conducted with male mice.

2.2. PPI reflex test

An LE 118–4 Startle and Fear Interface (Panlab, Barcelona, Spain) was used to measure the acoustic startle response of mice. The procedure was essentially the same as described previously [35]. Background noise was set at 60 dB. Pulse-alone trials consisted of a single white noise burst (120 dB, 40 ms). Prepulse + pulse trials consisted of a prepulse of noise (20 ms at 75 dB) followed 100 ms after prepulse onset by a startling pulse (120 dB, 40 ms). PPI of the startle response was calculated using the formula: %PPI = 100 − (startle response for prepulse and pulse trials/startle response for pulse-alone trials) × 100.

2.3. Brain tissue preparation

Mice were sacrificed by cervical dislocation. Brains were removed and rinsed with ice-cold isotonic saline. The frontal cortex (17.65 ± 4.8 mg), striatum (17.11 ± 4.7 mg) and hippocampus (23.18 ± 4.5 mg) were quickly dissected out and stored at −80 °C until use. The tissue samples were homogenized using a Polytron® homogenizer (Kinematica AG, Lucerne, Switzerland) in 0.2 M perchloric acid (10 µl/mg of tissue) including 100 µM EDTA-Na_2 and 1 ng/µl (10 µl) isoproterenol (Tokyo Company Industry Ltd., Japan) as an internal standard (IS). Samples were kept on ice for 30 min and were then centrifuged at 15,000 × *g* at 4 °C for 15 min. The supernatants were filtered through a sterile Sartorius Minisart 0.45-µm filter to remove the cells. A small amount of Na-acetate was added slowly to give the desired pH of about 3.0; 10 µL of the resulting solution were injected into the HPLC-ECD to determine the levels of monoamines and their metabolites.

2.4. HPLC-ECD conditions

We used an HPLC system equipped with an ECD-300 (Eicom, Japan) to determine *ex vivo* monoamines and their metabolites simultaneously in the brain. The main operating conditions for HPLC were: column: EicompaK SC-5ODS (3.0 mm × 150 mm), oven temperature 25 °C, detector, oxidation potential (+750 mV versus Ag/AgCl reference analytical electrode), mobile phase: 83 % citrate-acetate buffer (pH 3.5) containing 17 % methanol, 170 mg/l sodium octane sulfonate and 5 mg/l EDTA-Na_2, flow rate 0.23 ml/min. Samples were collected for 30 min. The chromatograms were recorded with a PowerChrom (AD Instruments, Sydney, Australia). Stock standard solutions (STD, 1 ng/µl) of NE, DA, 5-HT, DOPAC, HVA, 3-MT and 5-HIAA were purchased from Eicom and stored at 4 °C until use. For each experiment day, STD and IS were freshly prepared in phosphate buffer (0.1 M, pH 3.5) at concentrations of 1 pg/µl and 1 ng/µl, respectively.

2.5. Statistical analysis

Data are expressed as the mean ± SEM. Statistical analyses were performed by using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey–Kramer test. The level of significance of the *post hoc* tests was set at $p < 0.05$. All analyses were conducted using GraphPad Prism software version 5.0 (GraphPad, La Jolla, CA, USA). A *p*-value < 0.05 was considered statistically significant.

3. Results

3.1. PPI response

We examined PPI in *Mdga1*-KO, *Mdga1*-HT and WT mice (Fig. 1). ANOVA showed that *Mdga1*-KO mice exhibited significant reduction of PPI response compared with *Mdga1*-HT ($p < 0.05$) and WT ($p < 0.05$)

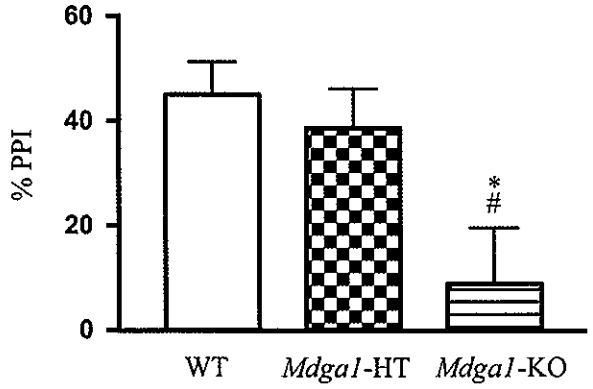

Fig. 1. PPI response in *Mdga1*-KO, *Mdga1*-HT and WT mice.
*p < 0.05 versus WT; # p < 0.05 versus *Mdga1*-HT. Data are expressed as mean ± SEM.

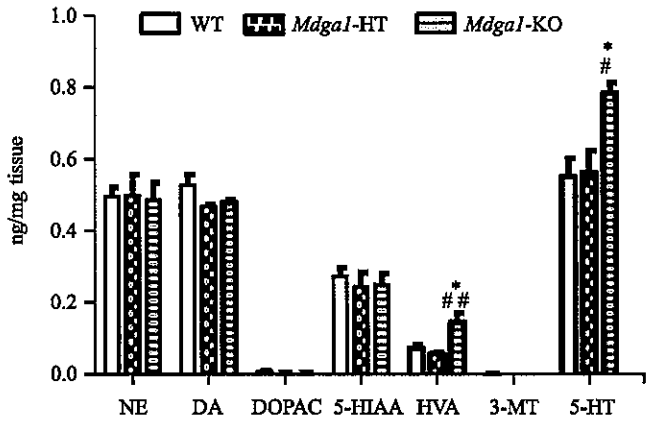

Fig. 2. Tissue content of monoamines and their metabolites in the frontal cortex.
*p < 0.05 versus WT; # p < 0.05 versus *MDGA1*-HT; ## p < 0.01 versus *Mdga1*-HT. Data are expressed as mean ± SEM. NE, Norepinephrine; DA, dopamine; 5-HT, serotonin; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine.

mice (df 2,24; F = 5.487, p < 0.05). *Mdga1*-HT mice showed no significant difference compared with WT mice.

3.2. Monoamines and their metabolites in the frontal cortex

Fig. 2 shows the differences in monoamines and their metabolite levels in the cortex of *Mdga1*-KO, *Mdga1*-HT and WT mice. ANOVA showed that *Mdga1*-KO mice had a significantly higher tissue content of the major DA metabolite HVA than *Mdga1*-HT (157.14 %; df 2,20; F = 7.66, p < 0.01) and WT (100 %; df 2,20; F = 7.67, p < 0.05) mice. *Mdga1*-KO mice also had significantly higher tissue 5-HT content than *Mdga1*-HT (31.32 %; df 2,20; F = 6.77, p < 0.05) and WT (32.02 %; df 2,20; F = 5.43, p < 0.05) mice. MDGA1-deficiency did not result in significant changes in NE, DA, DOPAC, 5-HIAA and 3-MT levels. There were no significant differences in DA, 5-HT and their metabolite levels between *Mdga1*-HT and WT mice.

3.3. Monoamines and their metabolites in the striatum

Fig. 3 shows the differences in monoamines and their metabolite levels in the striatum of *Mdga1*-KO, *Mdga1*-HT and WT mice. ANOVA showed that *Mdga1*-KO mice had significantly higher tissue content of

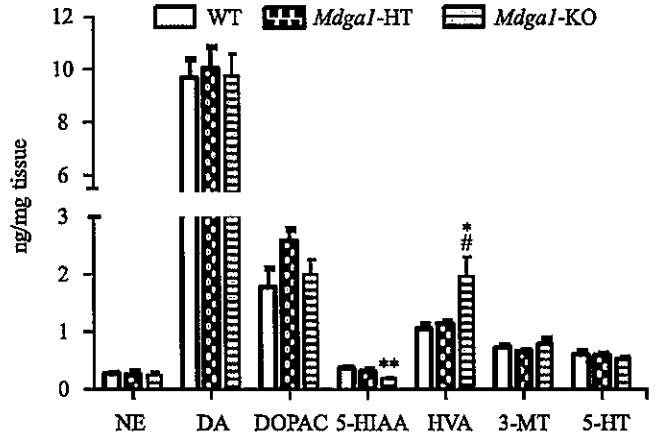

Fig. 3. Tissue content of monoamines and their metabolites in the striatum.
*p < 0.05 versus WT; ** p < 0.01 versus WT; # p < 0.05 versus *Mdga1*-HT. Data are expressed as mean ± SEM. NE, Norepinephrine; DA, dopamine; 5-HT, serotonin; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine.

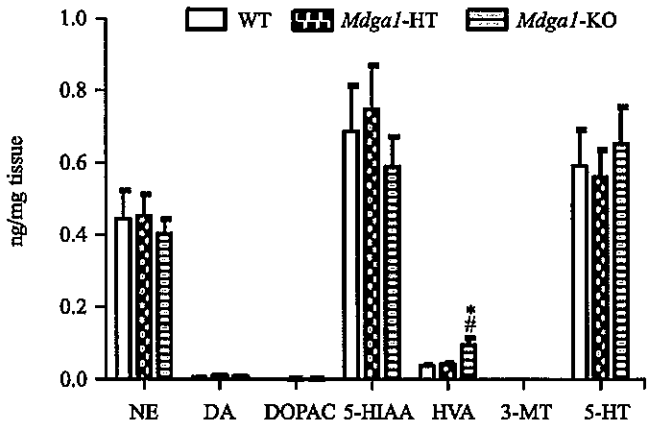

Fig. 4. Tissue content of monoamines and their metabolites in the hippocampus.
*p < 0.05 versus WT; # p < 0.05 versus *Mdga1*-HT. Data are expressed as mean ± SEM. NE, Norepinephrine; DA, dopamine; 5-HT, serotonin; DOPAC, 3,4-dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine.

the major DA metabolite HVA than *Mdga1*-HT (73.01 %; df 2,20; F = 5.71, p < 0.05) and WT (87.02 %, df 2,20; F = 5.71, p < 0.05) mice. *Mdga1*-KO mice had significantly lower levels of the major 5-HT metabolite 5-HIAA than WT (92.02 %; df 2,20; F = 7.74, p < 0.01) mice. *Mdga1*-deficiency did not result in significant changes in NE, DA, 5-HT, DOPAC and 3-MT levels. No significant difference in DA, 5-HT and their metabolite levels was observed between *Mdga1*-HT and WT mice.

3.4. Monoamines and their metabolites in the hippocampus

Fig. 4 shows the differences in monoamines and their metabolite levels in the hippocampus of *Mdga1*-KO, *Mdga1*-HT and WT mice. ANOVA showed that *Mdga1*-KO mice had significantly higher tissue content of the major DA metabolite HVA than *Mdga1*-HT (131.70 %; df 2,20; F = 6.15, p < 0.05) and WT (156.75 %, df 2,20; F = 6.15, p < 0.05) mice. *MDGA1*-deficiency did not result in significant changes in NE, DA, 5-HT, DOPAC, 5-HIAA and 3-MT levels. There were no significant differences in DA, 5-HT and their metabolite levels between *Mdga1*-HT and WT mice.

4. Discussion

Here, we examined PPI first to see whether there was any difference in acoustic startle response in *Mdga1*-KO mice, an animal model for schizophrenia and bipolar disorder [28,29], in comparison with the corresponding *Mdga1*-HT and WT mice. Second, we explored whether there were any differences in the tissue content of monoamines and their metabolites in the frontal cortex, striatum and hippocampus of *Mdga1*-KO mice, in comparison with *Mdga1*-HT and WT mice. This novel approach generated information on the impact of MDGA1 on monoamine systems. The major findings of this study are that *Mdga1*-KO mice exhibited reduced PPI, and had higher tissue content of HVA and lower tissue content of 5-HIAA than *Mdga1*-HT and WT mice. Our results in *Mdga1*-KO mice suggest that MDGA1 has an important role in PPI response and DA and 5-HT metabolism.

Research has shown that MDGA1-deficient mice exhibit cognitive deficits [21] associated with schizophrenia [36]. Reduced PPI response is another deficit observed in schizophrenic patients [37]. We found that *Mdga1*-KO mice exhibited significant reduction of PPI compared with *Mdga1*-HT and WT mice (Fig. 1), suggesting that MDGA1 deficiency can modulate PPI. The pathophysiology of schizophrenia involves dysfunctions in dopaminergic, serotonergic, and glutamatergic

neurotransmission; it is no surprise that manipulations of these systems have been the primary focus in animal models of PPI [32]. To address this issue, we used *Mdga1*-KO mice to measure *ex vivo* levels of DA, 5-HT and their metabolites in the brain.

HVA is a final product of DA metabolism and an indirect index of the change of DA turnover [38]. Determination of HVA concentration in brain tissue is, therefore, a suitable index for the evaluation of local DA turnover. There is an association between schizophrenic symptoms and DA overactivity; e.g., hyperactivity of subcortical and mesolimbic dopaminergic neurons has been proposed to produce symptoms including psychosis [39,40]. We measured NE, DA and its major metabolites DOPAC, 3-MT and HVA in the brains of *Mdga1*-KO, *Mdga1*-HT and WT mice. *Mdga1*-KO mice had significantly higher tissue content of HVA (an MAO- and COMT-dependent metabolite) in all three brain regions studied compared with *Mdga1*-HT and WT mice (Figs. 2–4). There were no changes in the levels of NE, DA, DOPAC and 3-MT in the brain regions analyzed. In schizophrenic patients, higher HVA levels have been reported compared with controls [41,42]. Higher tissue levels of HVA in *Mdga1*-KO mice may reflect increased DA function, which may contribute to development of symptoms of schizophrenia.

Degradation of DA, 5-HT and NE in the brain is essential for the correct functioning of synaptic neurotransmission [43]. COMT together with MAO catalyzes degradation of monoamines with COMT being responsible for the majority of DA and DOPAC catabolism in the brain. We may thus speculate that a high tissue content of HVA in *Mdga1*-KO mice is due to higher COMT activity than in *Mdga1*-HT and WT mice. In support of this notion, several studies have shown that the administration of COMT inhibitors produced a significant decrease in HVA levels in rat brain [44–46]. However, further experiments are needed to clarify this point. In our study, DA, DOPAC, HVA and 3-MT levels varied from high to undetectable in the striatum, cortex and hippocampus, respectively. The striatum normally contains high levels of DA and its metabolites [47]. DA in the cerebral cortex may be released other than from dopaminergic terminals, e.g. from noradrenergic ones, where DA acts as a NE precursor [48].

5-HIAA is the primary metabolite of 5-HT, and its levels in the brain give an indication of the turnover of 5-HT. Deficiency of 5-HT is thought to cause depression and anxiety [49–51]. A low level of 5-HIAA has been observed in CSF of patients with impulsive-aggressive personality disorders; thus, they may also have 5-HT deficiency [52]. A negative association between 5-HIAA level and aggressive behavior has been observed in rats [53], mice [54] and macaques [55]. We measured 5-HT and 5-HIAA in the brains of *Mdga1*-KO, *Mdga1*-HT and WT mice. Our results show that *Mdga1*-KO mice had lower levels of 5-HIAA (an MAO-dependent metabolite of 5-HT) in the striatum (Figs. 2–4). However, 5-HT levels remained stable in the brains of *Mdga1*-KO mice with the exception of a significant increase in 5-HT levels in the cortex (Figs. 2–4). The reason for these discrepant findings is not clear, but could be related to brain-region-specific *Mdga1* expression differences [19–21]. There is some evidence that high or low brain MAO activity might reflect high or low 5-HT turnover [56]. For example, lack of MAO resulted in a decrease of 5-HIAA in mouse brain [57]. Our results suggest that the observed low tissue content of 5-HIAA in *Mdga1*-KO mice is probably due to lower MAO activity than in *Mdga1*-HT and WT mice. Further experiments are needed to clarify this point. There were no significant changes of 5-HT and 5-HIAA tissue levels between *Mdga1*-HT and WT mice. The data reported in this study are the first evidence of serotonergic abnormalities in *Mdga1*-KO mice.

5. Conclusion

We report for the first time deficit in PPI and perturbation in DA and 5-HT metabolism in the brains of *Mdga1*-KO mice, suggesting a potential role of MDGA1 in modulating PPI and the metabolism of monoamine neurotransmitters. However, many aspects remain to be fully elucidated, and further studies are needed to address the role of the activity of multiple enzymes involved in the synthesis and metabolism of DA and 5-HT.

Declaration of Competing Interest

The authors declare no conflict of interest.

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