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Short Communication

Increased expression of soluble epoxide hydrolase in the brain and liver from patients with major psychiatric disorders: A role of brain – liver axis

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ABSTRACT

Background: Soluble epoxide hydrolase (sEH) in the metabolism of polyunsaturated fatty acids might play a role in the pathogenesis of major psychiatric disorders. Here we studied whether expression of sEH protein is altered in the postmortem samples (parietal cortex, and liver) from patients with major psychiatric disorders.
Methods: Protein expression of sEH in the parietal cortex and liver from control, major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia (SZ) groups was measured.
Results: Levels of sEH in the parietal cortex and liver from MDD, BD, and SZ groups were significantly higher than the control group. Interestingly, there was a positive correlation between sEH protein in the parietal cortex and sEH protein the liver in all groups.
Limitations: The small number in each group may limit our interpretation.
Conclusions: This study shows that the increased expression of sEH in the brain and liver might play a role in the pathogenesis of major psychiatric disorders, suggesting a role of brain – liver axis in major psychiatric disorders.

1. Introduction

Major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia (SZ) are major psychiatric disorders with complex etiologies. Postmortem brain tissue from patients with these psychiatric disorders is an underutilized substance that may be used to translate genetic and/or preclinical studies (McCullumsmith et al., 2011; Mechawar and Savitz, 2016; Vornholt et al., 2019).

Accumulating evidence suggests that abnormalities in the soluble epoxide hydrolase (sEH) in the metabolism of polyunsaturated fatty acids (PUFAs) play a key role in inflammation which is implicated in the pathogenesis of major psychiatric disorders (Hashimoto, 2019a; Hashimoto, 2019b; Swardfager et al., 2018). It is well known that sEH limits tissue levels of cytochrome P450 epoxides derived from ω − 6 and ω − 3 PUFAs by converting these anti-inflammatory mediators into their less active diols. Previously, we reported that sEH knock-out (KO) mice exhibit stress resilience after chronic social defeat stress, and that the sEH inhibitors have antidepressant-like effects in rodent models of depression (Ren et al., 2016). Furthermore, we reported a key role of sEH in the development of neurodevelopmental disorders (i.e., autism spectrum disorder, schizophrenia) in offspring after maternal immune activation (Ma et al., 2019). Collectively, it is likely that increased expression of sEH in the metabolism of PUFAs plays a role in the pathogenesis of inflammation-related psychiatric disorders (Hashimoto, 2019a; Hashimoto, 2019b; Hashimoto, 2019c). It is also known that the sEH protein is most highly expressed in the liver (Beetham et al., 1993; Gill and Hammock, 1980). However, there are no reports examining sEH expression in the liver from patients with major psychiatric disorders.

This present study was, therefore, undertaken to examine whether expression of sEH protein in postmortem samples (parietal cortex, and liver) taken from major psychiatric disorders including MDD, BD, and

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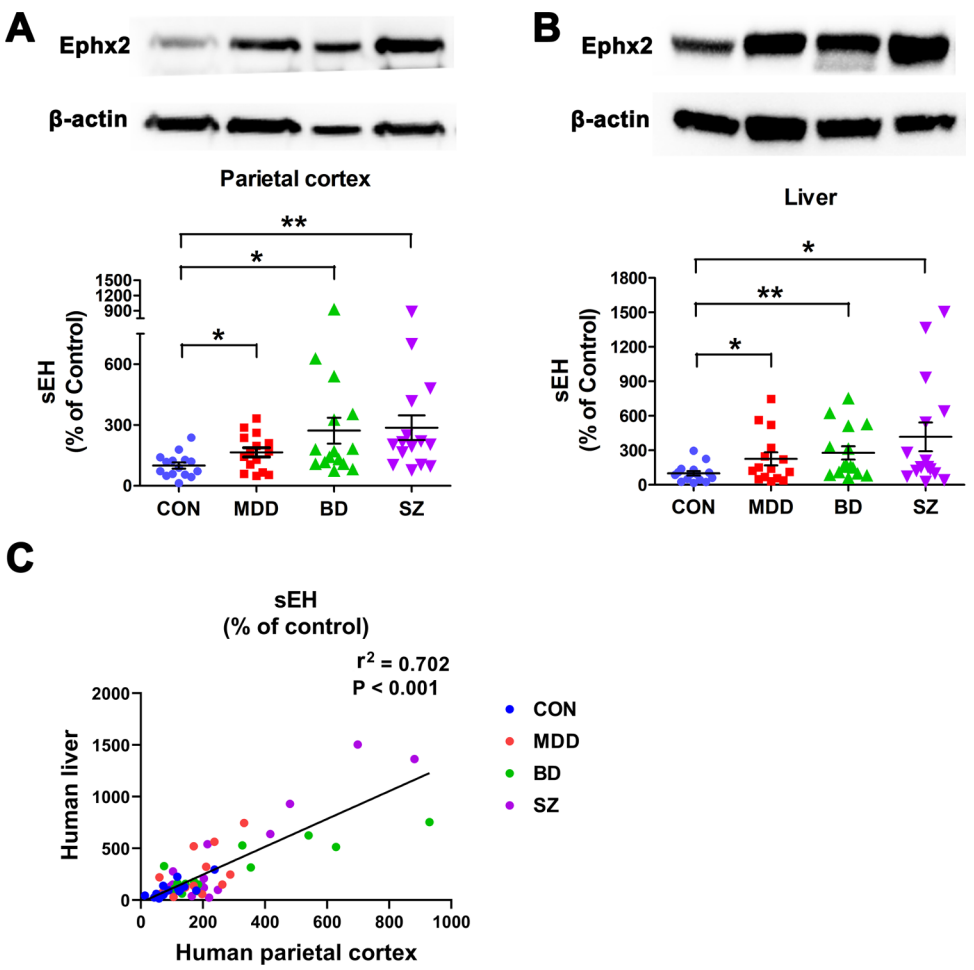

Fig. 1. Levels of sEH in the parietal cortex and liver from control, MDD, BD, and SZ groups. (A): Western blot analysis of sEH and β-actin in the parietal cortex of control (n = 15), MDD (n = 15), SZ (n = 15) and BD (n = 15) groups was performed. (B): Western blot analysis of sEH and β-actin in the liver of control (n = 15), MDD (n = 15), BD (n = 15) and SZ (n = 15) groups was performed. The representative bands of sEH and β-actin in the parietal cortex (A) and liver (B). The data are expressed as a percentage of control group values. The data are shown as mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the control group. (C): There was a positive correlation (r^2 = 0.702, $P < 0.001$) between sEH in the parietal cortex and sEH in the liver in the all groups (n = 60).

SZ showed differences when compared with a control group. Previously, we found a negative correlation between brain-derived neurotrophic factor (BDNF) in the parietal cortex and BDNF in the liver, suggesting a possible role of brain – liver axis in these psychiatric disorders (Yang et al., 2017). Therefore, we also examined the correlations between sEH protein in the parietal cortex and sEH protein in the liver.

2. Method

2.1. Postmortem human samples

Human postmortem parietal cortex (Brodmann area 7) and liver from normal controls (N = 15), as well as patients with major depressive disorder (MDD) (N = 15), bipolar disorder (BD) (N = 15), and schizophrenia (SZ) (N = 15) were obtained from the Stanley Foundation Brain Collection (Bethesda, MD, USA). The specimens were collected by medical examiners. Permission from the next of kin was obtained in all cases. The demographic, clinical, and storage information for cases has been previously published (Torrey et al., 2000). Each diagnostic group was matched according to several parameters, including age at death, gender, postmortem interval (PMI), brain pH, and brain weight (Hashimoto et al., 2007; Yang et al., 2017; Ren et al., 2016; Xiong et al., 2018; Zhang et al., 2018). This study was approved by the Research Ethics Committee of the Graduate School of Medicine, Chiba University.

2.2. Western blot analysis

Western blot analysis was performed by one observer who was blinded to the four groups. Human brain samples were stored at −80 °C until biochemical analyses. Tissue samples were homogenized in Laemmli lysis buffer, then centrifuged at 3000 × g at 4 °C, for 10 min to obtain the supernatants. Protein concentrations were determined using a BCA method assay kit (Bio-Rad, Hercules, CA), then samples were incubated for 5 min at 95 °C, with an equal volume of 125 mM Tris/HCl, pH 6.8, 20% glycerol, 0.1% bromophenol blue, 10% β-mercaptoethanol and 4% sodium dodecyl sulfate. Proteins were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, on 10% mini-gels (Mini-PROTEAN® TGX™ Precast Gel; Bio-Rad). Separated proteins were then transferred onto polyvinylidene difluoride membranes using a Trans Blot Mini Cell (Bio-Rad). For immunodetection, blots were blocked with 5% skim milk in TBST (TBS + 0.1% Tween-20) for 1 h at room temperature (RT), then incubated with primary antibodies overnight, at 4 °C. The following primary antibodies were used: anti-sEH (1:5000, prepared at UC Davis, CA) and β-actin (1:10,000, Sigma-Aldrich Co., Ltd., St Louis, MO, USA). The next day, blots were washed three times in TBST and incubated with horseradish peroxidase conjugated anti-rabbit or anti-mouse antibody (1:5000) for 1 h, at RT. After three washes in TBST, bands were detected using enhanced chemiluminescence (ECL), plus the Western Blotting Detection system (GE Healthcare Bioscience). Finally, blots were washed three times in TBST and incubated with a primary antibody directed against β-actin. Images were captured with a Fuji LAS3000-mini imaging system (Fujifilm, Tokyo, Japan), and immunoreactive bands were quantified.

2.3. Statistical analysis

The data are shown as the mean ± standard error of the mean (S.E.M). Analysis was performed using PASW Statistics 20 (formerly

SPSS statistics; SPSS, Tokyo, Japan). Analyses of covariance (ANCOVA) was performed on normalized spot volumes, for each spot in each region, with brain pH, age of disease onset, gender, duration of disease, postmortem interval (PMI), frozen brain hemisphere side, lifetime neuroleptic drug use, severity of substance abuse, severity of alcohol abuse, and/or frozen storage time. If ANCOVA reached significance, we performed the *post-hoc* Student Neuman-Keuls multiple comparison test. Correlation was determined by Pearson correlation. A *P*value of less than 0.05 was considered to be statistically significant.

3. Results

Since the postmortem samples have many parameters, the ANCOVA has been used for statistical analyses of the data. The ANCOVA of the data of sEH protein from the parietal cortex showed the statistical results [$F_{3,56} = 3.054$, $P = 0.037$] from the four groups. A *post-hoc* analysis showed that expression of sEH in MDD, BD, and SZ groups was significantly higher than in the control group (Fig. 1A).

Furthermore, The ANCOVA of the data of sEH protein from the liver showed the statistical results [$F_{3,56} = 2.895$, $P = 0.044$] from the four groups. A *post-hoc* analysis showed that expression of sEH in MDD, BD, and SZ groups was significantly higher than in the control group (Fig. 1B).

Next, we analyzed the correlation between sEH protein in the parietal cortex and sEH protein in the liver from all four groups. There was a positive correlation ($r^2 = 0.702$, $P < 0.001$) between sEH protein in the parietal cortex and sEH protein in the liver in the all groups (Fig. 1C).

We analyzed the effects of gender, age at death, age of disease onset, disease duration, lifetime neuroleptic drug use for the data of sEH. However, we did not find any significant effect for the outcome.

4. Discussion

In this study, we found that the tissue levels of sEH in the parietal cortex from MDD, BD, and SZ groups were significantly higher than those of the control group, consistent with the previous report (Ren et al., 2016). Furthermore, the tissue levels of sEH in the liver from MDD, BD, and SZ groups were also significantly higher than those of the control group. Interestingly, there was a positive correlation between sEH protein in the parietal cortex and sEH protein in the liver from the four groups. Thus, this strong correlation brings up the concept that the inflammation associated with these psychiatric disorders may be systemic and not just associated with a brain region. A recent study showed that C-reactive protein in the blood is associated with severity of thought and language dysfunction in patients with schizophrenia (Chang et al., 2019). These data suggest inflammatory events in the parietal cortex and liver from these psychiatric disorders. Therefore, it is likely that abnormalities in sEH-related PUFAs metabolism may play a role in the pathophysiology of these major psychiatric disorders, suggesting a new role of brain – liver axis.

The parietal cortex, one of four major lobes in the cerebral cortex of the human brain, plays important roles in integrating sensory information from various parts of the body. In this study, we found increased expression of sEH in parietal cortex from three psychiatric groups, consistent with previous report (Ren et al., 2016). Increased levels of sEH in the parietal cortex of these psychiatric disorders may cause inflammatory event in this region. Collectively, it is likely that abnormality in the PUFAs metabolism due to increased sEH expression in the parietal cortex may be involved in these psychiatric disorders, although further studies on the role of sEH in these psychiatric disorders are needed.

It is known that neurological and neuropsychiatric symptoms are associated with liver disease (Weissenborn et al., 2005). A population-based cohort study found that BD and SZ patients showed a significantly higher prevalence and incidence of chronic liver disease than the general population, and that even younger patients had a much higher prevalence and incidence of liver disorders than the general population (Hsu et al., 2014; 2016), suggesting a possible link between liver disease and BD and SZ. Furthermore, the high expression of sEH in liver suggests that hepatic sEH is a major contributor to systemic levels of anti-inflammatory fatty acids. A recent study demonstrated that hepatic deletion of sEH results in antidepressant-like effects, while the hepatic overexpression of sEH induces depression-like phenotypes in mice, suggesting a brain–liver axis (Qin et al., 2019). Given the postulated role for the brain–liver axis (Mighiu et al., 2012; Qin et al., 2019; Yang et al., 2017), it is likely that increased levels of sEH in liver tissue might contribute to the higher inflammatory event in the liver, resulting in high incidence of liver disease in these psychiatric disorders. Nonetheless, further detailed studies of the underlying association between abnormalities in the metabolism of PUFAs in the brain–liver axis and major psychiatric disorders are needed.

The current data suggest that the sEH could be a useful marker for inflammation involved in these psychiatric disorders. Although no causation is addressed in this study, our previous studies (Ren et al., 2016; Ma et al., 2019) showing that pharmacological inhibition or genetic knockout of the sEH prevents or reverse symptoms. Collectively, it is likely that the sEH could be a target for the development of prophylactic and therapeutic drugs.

In conclusion, this study suggests that increased expression of sEH in the parietal cortex and liver might play a role in the pathogenesis of major psychiatric disorders. Further detailed investigations on the role of brain – liver axis in the metabolism of PUFAs in major psychiatric disorders are warranted.

Declaration of Competing Interest

All authors report no biomedical financial interests or potential conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jad.2020.03.070.

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