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Decreased Prostaglandin D$_2$ Levels in Major Depressive Disorder Are Associated with Depression-Like Behaviors

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Abstract

Background: Prostaglandin (PG) D$_2$ is the most abundant prostaglandin in the mammalian brain. The physiological and pharmacological actions of PGD$_2$ in the central nervous system seem to be associated with some of the symptoms exhibited by patients with major depressive disorder. Previous studies have found that PGD$_2$ synthase was decreased in the cerebrospinal fluid of major depressive disorder patients. We speculated that there may be a dysregulation of PGD$_2$ levels in major depressive disorder.
Methods: Ultra-performance liquid chromatography-tandem mass spectrometry coupled with a stable isotopic-labeled internal standard was used to determine PGD$_2$ levels in the plasma of major depressive disorder patients and in the brains of depressive mice. A total of 32 drug-free major depressive disorder patients and 30 healthy controls were recruited. An animal model of depression was constructed by exposing mice to 5 weeks of chronic unpredictable mild stress. To explore the role of PGD$_2$ in major depressive disorder, selenium tetrachloride was administered to simulate the change in PGD$_2$ levels in mice.
Results: Mice exposed to chronic unpredictable mild stress exhibited depression-like behaviors, as indicated by reduced sucrose preference and increased immobility time in the forced swimming test. PGD$_2$ levels in the plasma of major depressive disorder patients and in the brains of depressive mice were both decreased compared with their corresponding controls. Further inhibiting PGD$_2$ production in mice resulted in an increased immobility time in the forced swimming test that could be reversed by imipramine.
Conclusion: Decreased PGD$_2$ levels in major depressive disorder are associated with depression-like behaviors.

Keywords: prostaglandin D$_2$, major depressive disorder, mice, patients, depression-like behaviors

Introduction

Major depressive disorder (MDD) is one of the most common and debilitating mental disorders, demonstrating high prevalence and mortality (Kessler et al., 2003; Kessler and Bromet, 2013). It is characterized by episodes of depressed mood or a loss of interest or pleasure in daily activities for more than 2 weeks (Nestler et al., 2002). MDD occurs frequently and causes a heavy burden on society (Sartorius, 2001). The World Health Organization (WHO) has projected that MDD will be the second leading cause of disability by the year 2020 (Murray and Lopez, 1996). However, despite a variety of hypotheses proposed over

Significance Statement

Prostaglandin (PG) D_2 is a biologically active lipid mediator that is widely distributed in the peripheral tissues and central nervous system (CNS). In the CNS, PGD_2 acts as a neuromodulator. Research regarding the role of PGD_2 in the development of major depressive disorder (MDD) is limited. Here, using a method with ultra-performance liquid chromatography-tandem mass spectrometry coupled with a stable isotopic labeled internal standard, we found that PGD_2 levels in the plasma of MDD patients and in the brains of depressive mice were both decreased. Further inhibition of PGD_2 production led to behavioral despair in the forced swimming test.

the past several years (Massart et al., 2012; Cai et al., 2015), the underlying pathophysiological mechanism of MDD remains poorly understood.

Prostaglandins (PGs) are a group of biologically active lipid mediators derived from the cyclooxygenase pathway of the arachidonic acid cascade (Simmons et al., 2004). They are widely distributed in peripheral tissues and the central nervous system (CNS). PGs can either directly or indirectly influence neuronal activity and participate in the regulation of physiological and pathophysiological processes in the CNS (Wolfe and Coceani, 1979). Evidence has shown that alterations in PG metabolism are closely associated with mental disorders (Gross et al., 1977; Sublette et al., 2004). For example, PGE_2 is reported to mediate the attenuation of mesocortical dopaminergic pathway, which is critical for susceptibility to repeated social defeat stress in mice (Tanaka et al., 2012). And inhibition of COX-2, which catalyzes synthesis of inflammatory PGs, is shown to reduce stress-induced affective pathology (Gamble-George et al., 2016).

PGD_2 is the most abundant PG in the mammalian brain (Narumiya et al., 1982; Ogorochi et al., 1984). It is synthesized from the cyclooxygenase product PGH_2 by the action of PGD_2 synthase (PGDS) (Urade, 2008). Two distinct types of PGDS have been identified: the lipocalin-type PGDS (L-PGDS) and the hematopoietic-type PGDS. L-PGDS is mainly localized in the leptomeninges and choroid plexus. It is responsible for the biosynthesis of PGD_2 in the brain (Urade and Hayaishi, 2000). Selenium tetrachloride ($SeCl_4$) has been reported to specifically inhibit PGD_2 production by interacting with L-PGDS in vivo (Qu et al., 2006; Gonzalez-Rodriguez et al., 2014). In the central nervous system (CNS), PGD_2 has many functions such as neuronal protection (Liang et al., 2005), temperature regulation (Ueno et al., 1982), the induction of non-rapid eye movement sleep (Urade and Hayaishi, 2011), and the attenuation of pain response (Eguchi et al., 1999). Additionally, via the DP1 receptor, PGD_2 stimulates food intake (Ohinata et al., 2008) and exhibits anxiolytic-like activity (Zhao et al., 2009). Combining the protective roles displayed by PGD_2 with the discovery that L-PGDS was decreased in the cerebrospinal fluid of MDD patients (Ditzen et al., 2012), we hypothesized the existence of PGD_2 dysregulation in MDD.

In this study, to explore whether PGD_2 plays a role in the development of MDD, we detected PGD_2 levels in the plasma of MDD patients and in the brains of depressive mice. Furthermore, by using a pharmacological method, we assessed the effects of PGD_2 dysregulation on depression-like behaviors in mice.

Materials and Methods

Experimental Design

We first investigated PGD_2 levels in the plasma of MDD patients. Then we constructed an animal model of depression by exposing mice to chronic unpredictable mild stress (CUMS) and assessed the content of PGD_2 in the mice brains. Considering

the roles of PGD_2 in the CNS, we further examined the effects of PGD_2 reduction on depression-like behaviors in mice by administrating $SeCl_4$. Moreover, the depression-like behavior induced by decreased PGD_2 was validated by administrating one of the classic antidepressants, imipramine. Depression-like behaviors in mice were evaluated by open field test (OFT), sucrose preference test (SPT), and forced swimming test (FST). Grip strength test (GST) was performed to exclude the possibility that the immobility of mice in the FST induced by $SeCl_4$ was due to muscle dysfunction.

Participants and Samples

Unrelated patients (n = 32, age range 17–60 years) diagnosed with MDD using the Structured Clinical Interview for DSM-IV were recruited from the Department of Psychiatry, First Hospital of Shanxi Medical University, Taiyuan, China. All participants were first-episode patients. Patients' depression severity was assessed by at least 2 consultant psychiatrists according to the 17-item Hamilton Depression Rating Scale (HDRS). Patients were divided into anxious and nonanxious depression. Anxious depression was defined as MDD with high levels of anxiety symptoms, as reflected in a HDRS anxiety/somatization factor score ≥7. The anxiety/somatization factor included 6 items from the 17-item HDRS: the items for psychic anxiety, somatic anxiety, gastrointestinal somatic symptoms, general somatic symptoms, hypochondriasis, and insight (Fava et al., 2008). Healthy controls (n = 32, age range 25–45 years) without a family history of psychosis in the prior 2 generations were recruited through posted advertisements. All the participants were of the Chinese Han origin and geographically came from northern China. None of the patients or healthy controls enrolled in this study showed a history of substance dependence or abuse, nor had any of the participants taken any psychotropic medications or nonsteroidal antiinflammatory drugs within 4 weeks. All the participants provided written informed consent, and the study was approved by the Ethics Committee of the Chinese Academy of Medical Sciences and Peking Union Medical College.

Peripheral venous blood samples were collected between 8:00 AM and 10:00 AM from participants in a resting state, following a 12-h overnight fast. Whole blood was collected into chilled EDTA-treated vacutainer tubes and placed on wet ice. The plasma was promptly separated by centrifugation (1500 × rpm at 4°C for 10 minutes) and aliquoted into polypropylene tubes. All samples were stored at -80°C until analysis.

Animals

Male 8- to 12-week-old C57BL/6J mice (Vital River) weighing 22- to 6 g were housed in plastic cages with free access to food and water ad libitum under standard conditions (12-h-light-/dark cycle; lights on from 8:00 AM to 8:00 PM; 22 ± 2°C ambient temperature; 55 ± 10% relative humidity). All experimental

procedures were approved by the Animal Ethics Committee of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, and were conducted in accordance with the institutional guidelines for animal care and use set by the Beijing Administration Office of Laboratory Animals.

Chronic Unpredictable Mild Stress (CUMS) Model

Upon arrival, wild-type C57BL/6J mice were allowed to acclimate to the experimental environment for 1 week. Body weights and basic sucrose preferences of all animals were measured before they were separated into 2 groups (10/group): the experimental group and the control group. The control group was housed in normal conditions and manipulated once a week only for cage cleaning, whereas the experimental group was subjected to the CUMS model for 5 weeks. The CUMS procedure was performed according to the protocol described previously (Willner, 2005) with minor modifications. The unpredictable mild stressors used in this study included restraint (in 50-mL cylindrical plastic tubes with holes for air flow, 1 hour), food and water deprivation (12 hours), crowded housing (10 animals per cage, 12 hours), cage tilting (45°,12 hours), soiled bedding (pour water into sawdust bedding, 12 hours), removal of sawdust (12 hours), an elevated Plexiglas platform (1 m tall, 21 × 21 cm, 30 minutes), a hot stimulus (45℃ in a square transparent Plexiglas box placed on an electric blanket, 10 minutes), overnight illumination, and reversed light/dark cycle. All the stressors were randomly applied throughout the 5-week experiment, and the same stressor appeared at least every 2 days to avoid animals' habituation. After 5 weeks of CUMS, behavioral tests were performed in order. Then the mice were sacrificed for sample collection. The whole brain of each animal was removed and quickly washed in ice-cold PBS and frozen in liquid nitrogen before transfer to -80°C for storage. The timeline of the CUMS procedure and sample collection was shown as Figure 2A.

Behavioral Tests

All behavioral tests were conducted during the light phase. Before the tests, mice were introduced to the experiment room to acclimate to the environment for at least 2 hours. Behavioral tests were performed by slightly modifying the methods reported earlier (Xu et al., 2016).

OFT

The apparatus used in the OFT was an open box (50 cm x 50 cm x 50 cm) with a Plexiglas floor that was divided into 16 equal-sized squares. The squares were further subdivided into a central zone with 4 inner squares and a peripheral zone with the other 12 squares close to the wall. Each mouse was placed individually into the central zone at the beginning of the test and allowed to move freely for a total time of 5 minutes. The locomotor activity of the mice was monitored and traced with an automated video-tracking system (Ethovision 9.0, Noldus) as previously described (Prut and Belzung, 2003). The apparatus was thoroughly cleaned with 75% ethanol followed by water between each test.

SPT

All the mice experienced 2 SPTs. The first test was to examine the basic sucrose preference of the mice and the second test was to examine the effects of the experimental factors. Before the first test there was a habituation phase. During the habituation phase, mice were housed in groups and were trained to become acclimated to drinking from 2 identical bottles: one containing 1% sucrose solution (w/v) and the other containing tap water. This phase persisted for 48 hours (the sucrose and water bottles switched ever 24 hours to avoid position preference). Once the habituation phase ended, the first SPT began. Mice were introduced to the experimental room housing individually and were deprived of food and water for 12 h. Then, a 2-hour test of sucrose preference was performed. During the test, mice were presented simultaneously with 2 identical bottles, one containing 1% sucrose solution (w/v) and the other containing tap water. The positions of the bottles were switched every 1 hour. Sucrose consumption and water consumption were measured by comparing the weights of the bottles before and after the test. Sucrose preference was shown by calculating the ratio of sucrose consumption to the total consumption of sucrose and tap water. Mice with a basic sucrose preference below 65% were excluded from the CUMS procedure (Strekalova et al., 2011).

FST

The FST was carried out following the protocoal of Rupniak et al. (Rupniak et al., 2001). Briefly, mice were individually forced to swim in transparent glass cylinders (diameter 14 cm, height 20 cm) containing 15 cm of water at 25 ± 1°C. The swimming behaviors of each mouse were video-recorded for 6 minutes. The latency to the first episode of immobility and the total duration of immobility during the last 4 minutes were scored. Immobility was defined as no movement or passively floating with only enough movement to keep the head above water.

GST

The GST was conducted according to the manufacturer's instructions (Bioseb - In Vivo Research Instruments). Briefly, mice were held by the tail and raised above the grid. Mice were allowed to grasp the grid using their forelimbs and were then pulled backward following the axle of the sensor until they released the grid. The force achieved by the animal was displayed on the screen. The test was repeated 3 consecutive times within the same session, and the mean of all trials was recorded for grip strength for that animal.

Relative Quantitation of Plasma PGD_2 in Patients and Controls

Relative quantitation of plasma PGD_2 in patients and controls was completed with an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) based method (Zhang et al., 2015). Plasma PGD_2 was extracted by solid-phase extraction. Briefly, 250 µL of patient and control plasma samples were thawed on ice and mixed with 5 µL of 1% BHT (Cayman Chemicals) and PGD_2-d_4 (400 pg, Cayman Chemicals) before loading onto Oasis 10 mg HLB cartridges (Waters Co). Solid-phase extraction cartridges were conditioned and equilibrated according to the manufacturer's instructions and washed with 1 mL of 5% methanol (LC-MS grade, Fisher Scientific). After drying under a high vacuum for 20 minutes, PGD_2 was eluted with 1 mL of methanol and evaporated to dryness under a gentle stream of nitrogen at room temperature. Samples were reconstituted in 40 µL of acetonitrile/water (30:70) (LC-MS grade, Fisher Scientific) prior to injection into the LC-MS/MS system (AB SCIEX QTRAP 5500 triple quadrupole mass spectrometer equipped with a Turbo electrospray ionization source, Shimadzu Prominence

LC-20AD UFLC system, and a CTC autosampler). The detailed chromatographic and mass spectrographic parameters can be found in the supplementary Methods and Materials.

PGD_2 and PGE_2 Quantitation in the Mouse Brain

Cervical dislocation was performed to kill mice before brain sampling, except for those mice used in the CUMS procedure. Mice used in the CUMS procedure, including the control group, were sacrificed for brain sampling under deep anesthesia with chloral hydrate (500 mg/kg, Sangon Biotech). The protocol of PGD_2 and PGE_2 quantitation in the mouse brain has been described in detail previously (Masoodi and Nicolaou, 2006). A SepPak tC18 40 mg 96-well plate (Waters Co.) was used for the solid phase extraction of the 2 prostaglandins in this study. Simply, the whole brain of each mouse was weighed and homogenized in water (LC-MS grade, Fisher Scientific) with a superfine homogenizer (Fluko, Shanghai, China). The homogenization process was kept on ice. Next, 500 μL of homogenate containing 50 mg of brain tissue was transferred to a new Eppendorf tube and adjusted to 15% methanol (v/v) before mixing with 40 ng PGD_2-d_4 and PGE_2-d_4 (Cayman Chemicals). The mixture was centrifuged (4°C, 12 000 × rpm, 15 minutes) and acidified with 20 μL HAc (Amresco Inc.) to a pH of 3.0 and then placed on ice in the dark for 20 minutes. Immediately after, the acidified supernatant was loaded onto the 96-well plate that had been pretreated with 1 mL of methanol followed by 1 mL of water. The cartridges were then washed with 1.5 mL of 15% methanol, 1.5 mL of water, and 1 mL of hexane (HPLC grade, Sigma-Aldrich) in succession. Finally, the PGs were eluted with 1 mL of methyl formate (HPLC grade, Sigma-Aldrich) and dried under a gentle stream of nitrogen. The residues were reconstituted in 37 μL of acetonitrile/water (30:70).

Chromatographic separation and MS analysis were conducted according to the method used in the quantitation of plasma PGD_2. Calibration lines were prepared for the absolute quantitation of PGD_2 and PGE_2 in the brain (see supplementary Methods and Materials).

Drug Treatments

Selenium tetrachloride ($SeCl_4$, Sigma Aldrich) was dissolved in sterile saline. Aliquots were prepared and stored at -20°C. Before each experiment, an appropriate number of aliquots was taken to thaw at room temperature, and the pH was adjusted to 7.2 by the addition of 0.2 M NaOH. $SeCl_4$ was administered 2 hours before the behavioral tests. Imipramine hydrochloride (Sigma Aldrich) was freshly prepared in sterile saline each time before administration. The dose of imipramine (20 mg/kg) used in this study was previously reported to cause antidepressant activity in the forced swimming test (Popik et al., 2003; Garcia et al., 2008). Imipramine was given 30 minutes before the test. All drugs were delivered by i.p. injection. The control group was treated with an equal volume of saline.

Statistics

Data analysis was performed by using IBM SPSS Statistics (Version 22). For human participants, the chi-squared test was applied to test the differences in gender. Difference in age was analyzed by unpaired Student's t test. A 2-way ANOVA was performed to analyze PGD_2 levels with gender (male, female) and group (MDD, control) as between-subject factors. The anxiety/somatization subscale score did not follow a normal distribution, so a Mann-Whitney U test was used. For animals, data of the

behavioral tests were compared using unpaired Student's t test except for the data of the SPT in the CUMS procedure. The data was analyzed by Mann-Whitney U test. To examine the main effects of $SeCl_4$ (0, 1, and 2.5 mg/kg) on PGD_2 and PGE_2 levels in the mouse brain, a 1-way ANOVA followed by Bonferroni's post-hoc test was applied. A 2-way ANOVA was conducted to evaluate interactions between imipramine administration and $SeCl_4$ pre-treatment on mice immobility time in the FST. Specific methods of analysis are also described in the figure legends. All data are expressed as the mean ± SEM, and a value of $P < .05$ was considered significant.

Results

Sample Characteristics

As shown in Table 1, a total of 32 MDD patients (38.50 ± 12.1 years) and 30 healthy controls (34.13 ± 6.7 years) were recruited. There were no significant differences between controls and MDD patients in age, gender, race, or history of substance abuse. MDD patients were first-episode patients of depression. All the participants were free from psychotropic medications and had not taken any nonsteroidal antiinflammatory drugs within 4 weeks.

MDD patients were subdivided into anxious and nonanxious depression. As shown in Table 2, there were no significant differences between patients with anxious and nonanxious depression in age, gender, or family history of MDD. The anxiety/somatization factor score of patients with anxious depression was significantly higher than that of patients with nonanxious depression ($P < .001$).

Table 1. Demographic and Clinical Characteristics of Participants

Characteristic	MDD	Control	P value
n	32	30	
Age (y) (mean, SD)	38.50 (12.1)	34.13 (6.7)	.083[a]
Female/male (n)	20/12	16/14	.465[b]
Race	Han Chinese	Han Chinese	
History of substance abuse (n)	0	0	
Drug use within 4 weeks (n)	0	0	
HDRS–17 total score (mean, SD)	22.56 (3.3)		

Abbreviations: HDRS-17, 17-item Hamilton Depression Rating Scale; MDD, major depressive disorder.
[a]Unpaired Student's t test.
[b]Chi-squared test.

**Table 2.** Clinical Characteristics of MDD Patients

Characteristic	Anxious depression	Nonanxious depression	P value
n	21	11	
Age (y) (mean, SD)	38.71 (12.8)	38.09 (11.1)	.892[a]
Female/male (n)	12/9	8/3	.387[b]
Family history of MDD (n)	6	6	.149[b]
Anxiety/somatization subscale score (mean, SD)	8.76 (1.5)	5.36 (1.0)	<.001[c]

Abbreviation: MDD, major depressive disorder.
[a]Unpaired Student's t test.
[b]Chi-squared test.
[c]Mann-Whitney U test.
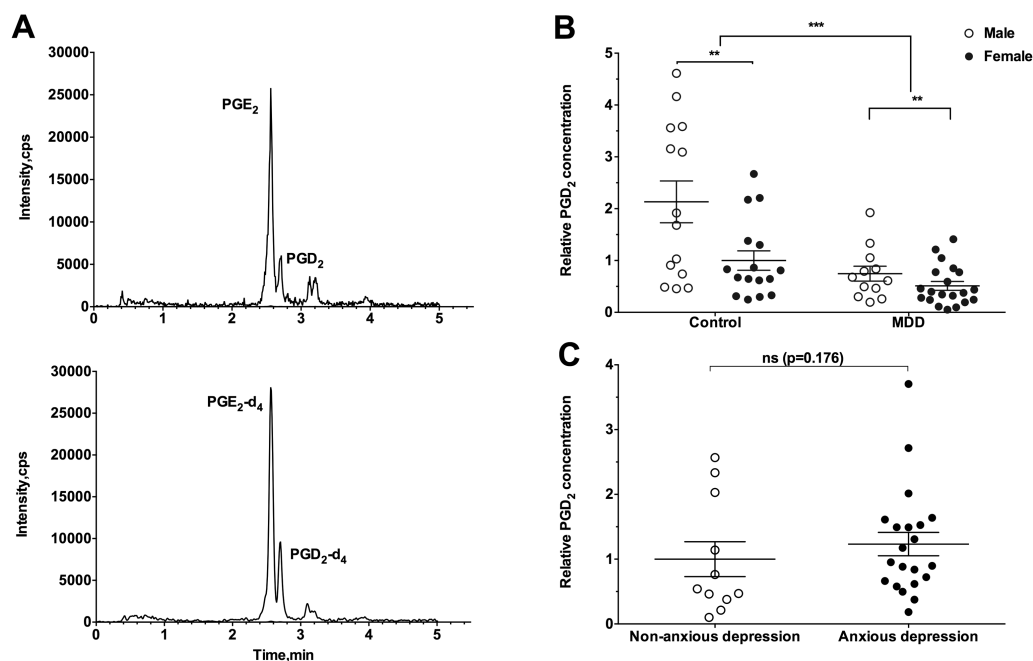
Figure 1. Reduction of plasma prostaglandin (PG)D$_2$ concentration in major depressive disorder (MDD) patients. (A) Representative chromatograms of PGD$_2$ and its stable isotopic internal standard PGD$_2$-d$_4$ in sample. (B) Analysis of the relative PGD$_2$ concentration in MDD patients and controls. The mean peak area ratio between the PGD$_2$ and PGD$_2$-d$_4$ of female controls was set as 1. Open and closed circles represent males and females, respectively. Two-way ANOVA. (C) Plasma PGD$_2$ concentration in patients with anxious and nonanxious depression. The mean peak area ratio between PGD$_2$ and PGD$_2$-d$_4$ of nonanxious depression was set as 1. Open and closed circles represent individual's PGD$_2$ concentration in patients with nonanxious and anxious depression, respectively. Unpaired Student's t test. Data are mean ± SEM. **$P < .01$, ***$P < .001$; ns, no significance.

Reduction of PGD$_2$ Levels in the Plasma of MDD Patients

The 2-way ANOVA revealed a significant effect of group ($F_{(1, 58)} = 16.20$, $P < .001$), a significant effect of gender ($F_{(1, 58)} = 8.17$, $P < .01$), but not a significant interaction between gender and group ($F_{(1, 58)} = 0.254$, $P = .596$). Figure 1B showed that plasma PGD$_2$ concentrations in MDD patients were significantly decreased compared with controls ($P < .001$), and plasma PGD$_2$ concentrations in males were significantly higher than females ($P < .01$). Because patients with MDD often also suffer from anxiety (Fawcett and Kravitz, 1983), and PGD$_2$ was shown to induce anxiolytic-like activity (Zhao et al., 2009), we divided the MDD patients into anxious and nonanxious depression to explore whether there existed a difference of plasma PGD$_2$ levels between the 2 groups. As illustrated by Figure 1C, there was no significant difference in plasma PGD$_2$ levels between patients with anxious and nonanxious depression ($P = .176$).

Depression-Like Behaviors and Decreased PGD$_2$ Levels in the Brains of Mice Exposed to CUMS

To explore whether the change of PGD$_2$ also existed in the CNS, we constructed an animal model of depression by exposing mice to CUMS and detected the PGD$_2$ levels in the mice brains. After 5 weeks of CUMS, depression-like behaviors of mice were assessed using the OFT, SPT, and FST. Compared with the control group, the CUMS mice showed a significant decrease in time spent in the central zone in the OFT ($P < .01$, Figure 2C), which indicated an anxiety-like behavior. No difference in locomotor activity was found between the 2 groups ($P = .692$, Figure 2B). The SPT was administered to evaluate anhedonia. There was no significant difference in basic sucrose preference between the control group and the CUMS group before the CUMS procedure ($P = .156$) (Figure 2D). Exposure to CUMS resulted in a significant

reduction of sucrose preference when compared with the non-stressed controls ($P < 0.01$) (Figure 2E). In the FST, the CUMS mice displayed behavioral despair reflected by significantly longer periods of immobility than the control group ($P < .05$) (Figure 2F). These results indicate that CUMS effectively causes depression-like behaviors in mice. PGD$_2$ levels in the brains of depressive mice were then investigated. Compared with the nonstressed controls, the mice subjected to CUMS demonstrated significant decreases in PGD$_2$ concentration in the brain ($P < .05$) (Figure 2G).

Inhibiting PGD$_2$ Production by an i.p. Injection of SeCl$_4$ Caused Behavioral Despair in Mice

We investigated the PGD$_2$ content in the brains of mice at 2 hours after an i.p. injection of SeCl$_4$ at a dose of 1 and 2.5 mg/kg body weight. As shown in Figure 3A, 2.5 mg/kg SeCl$_4$ significantly inhibited PGD$_2$ production ($P < .01$). No effect of SeCl$_4$ on PGE$_2$ content was found ($F_{(2, 15)} = 0.29$, $P = .752$) (Figure 3B).

To explore whether PGD$_2$ had a causal role in depression, we then examined the effects of 2.5 mg/kg SeCl$_4$ on depression-like behaviors in mice. The control mice were treated with an equal volume of saline. Compared with the control mice, the total distance traveled by the mice injected with 2.5 mg/kg SeCl$_4$ in the OFT was significantly decreased ($P < .01$, Figure 3C), but the time spent in the central zone showed no difference ($P = .848$, Figure 3D). In the SPT, there was no significant difference between the 2 groups treated with either 2.5 mg/kg SeCl$_4$ or saline ($P = .361$) (Figure 3F). However, an i.p. injection of SeCl$_4$ at 2.5 mg/kg did cause a phenotype of behavioral despair in mice, which was represented by significantly increased immobility time in the FST ($P < .05$) (Figure 3G). The GST was conducted to exclude the possibility that the depression-like behaviors induced by SeCl$_4$ in mice might be the result of muscle dysfunction. As shown in Figure 3H, no difference was found between mice injected with 2.5 mg/kg SeCl$_4$ and saline in the GST ($P = .851$).
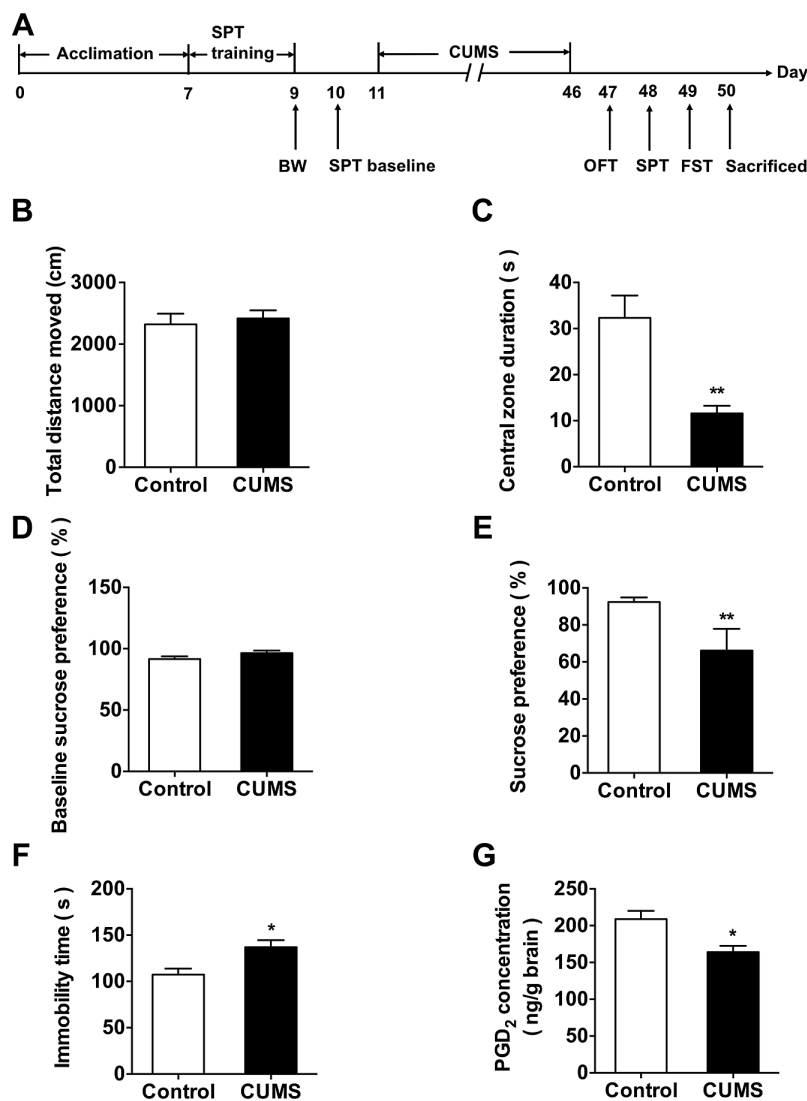
Figure 2. Depression-like behavior and decreased prostaglandin (PG)D$_2$ concentration in the brain of mice exposed to chronic unpredictable mild stress (CUMS). (A) A timeline of the CUMS procedure and sample collection. (B) Total distance moved and (C) total time spent in the central zone in the OFT. Unpaired Student's t test. (D) Sucrose preference before and (E) after CUMS in the sucrose preference test (SPT). Mann-Whitney U test. (F) Immobility time in the forced swim test (FST). Unpaired Student's t test. (G) PGD$_2$ concentration in the brain of mice. Unpaired Student's t test (control, n = 10; CUMS, n = 7). Data are mean ± SEM. *P < .05, **P < .01.

Imipramine Reversed SeCl$_4$-Induced Behavioral Despair in the FST

The effect of the classic antidepressant imipramine (20 mg/kg) on mice pretreated with 2.5 mg/kg SeCl$_4$ in the FST was assessed to validate the behavioral despair induced by SeCl$_4$ (Figure 4). The 2-way ANOVA revealed a significant effect of imipramine treatment ($F_{(1, 24)} = 13.83$, $P < .01$), a significant effect of SeCl$_4$ pretreatment ($F_{(1, 24)} = 7.43$, $P < .05$), and a significant interaction between imipramine treatment and SeCl$_4$ pretreatment ($F_{(1, 24)} = 5.37$, $P < .05$). Further simple main effects analysis showed that imipramine completely blocked the increase in immobility time elicited by SeCl$_4$ in the FST ($F_{(1, 24)} = 18.22$, $P < .001$).

Discussion

In the present study, with accessibility to a more sensitive and specific quantitative method, we found that the PGD$_2$ levels in the plasma of MDD patients and in the brains of depressive mice were both decreased compared with their corresponding controls. Simulation of this decreased PGD$_2$ in mice by pharmacologically inhibiting PGD$_2$ production led to depression-like behaviors.

PGD$_2$ is a relatively unstable molecule that can be degraded nonenzymatically to yield PGs of the J$_2$ series (Kikawa et al., 1984; Shibata et al., 2002), and it shares a very similar structure with other PGs such as PGE$_2$. Accurate quantification of PGD$_2$ in plasma has been a challenge for some time. As a result, the investigation of the role of PGD$_2$ in the pathophysiology of MDD may primarily depend on the sensitive and specific determination of PGD$_2$ content in the disease state. With use of stable isotopic labeled internal standard to correct for matrix effects, the UPLC-MS/MS-based method was reported to be the most accurate quantitative approach (Xu et al., 2007). In this study, using UPLC-MS/MS coupled with stable isotopic labeled internal standard PGD$_2$-d$_4$, we found that PGD$_2$ levels in the plasma of MDD patients were lower than those of healthy controls. And there was no significant difference in PGD$_2$ levels between patients with anxious and nonanxious depression. Our results are inconsistent with previous studies that have reported an
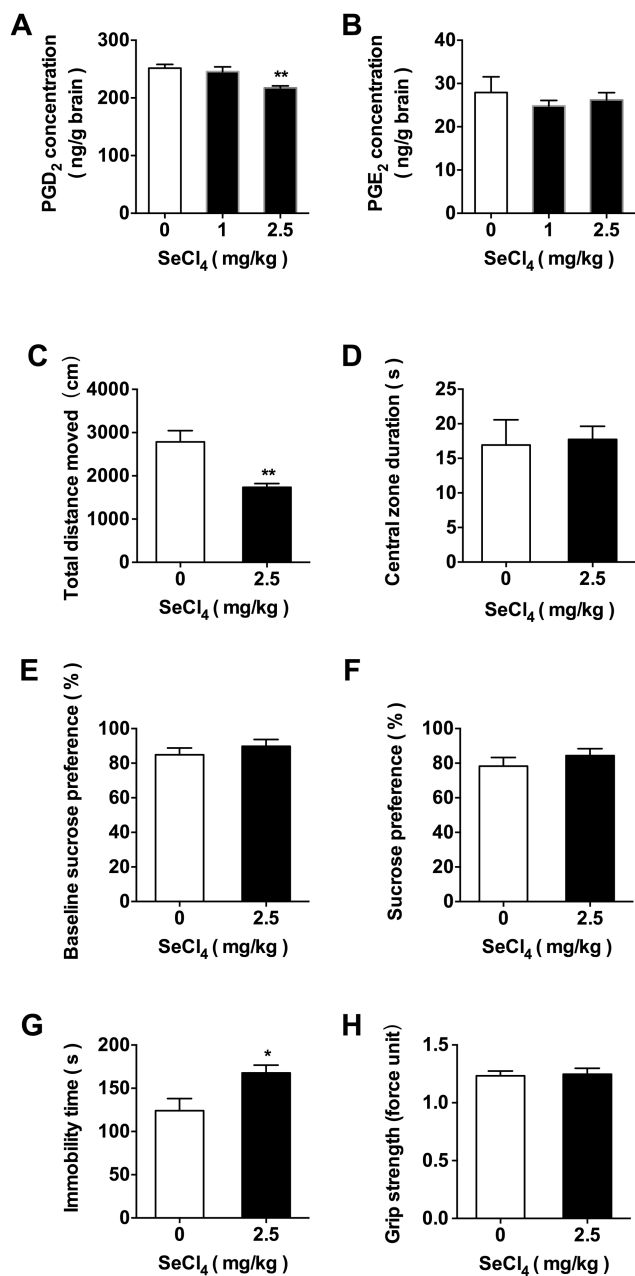
Figure 3. Inhibition of prostaglandin (PG)D$_2$ production and induction of behavioral despair by SeCl$_4$. (A) PGD$_2$ and (B) PGE$_2$ contents in the brain of mice after an i.p. injection of SeCl$_4$ at a dose of 1 and 2.5 mg/kg body weight for 2 hours. One-way ANOVA followed by Bonferroni's posthoc test (n = 6/group). (C) Total distance moved and (D) total time spent in the central zone in the open field test (OFT) after an injection of 2.5 mg/kg SeCl$_4$. (E) Sucrose preference before and (F) 2 hours after the injection of 2.5 mg/kg SeCl$_4$ in the sucrose preference test (SPT). (G) Immobility time in the forced swim test (FST) after an injection of 2.5 mg/kg SeCl$_4$. (H) Grip strength test (GST) after an injection of 2.5 mg/kg SeCl$_4$. Unpaired Student's t test (n = 8/group). Data are mean ± SEM. *$P < .05$, **$P < .01$. For each test, separate cohorts of mice were used.

increase in the level of salivary PGD$_2$ in major depression after implementing a radioimmunoassay for PGD$_2$ detection (Ohishi et al., 1988; Nishino et al., 1989). The different results may be mainly attributed to the different methods. As radioimmunoassay is an antibody-based method that may suffer from cross-reactivity and result in reduced selectivity (Tate and Ward, 2004).

Several lines of evidence indicate that PGs act through autocrine or paracrine means (Ashby, 1998; Jabbour et al., 2002); therefore, the reduction of PGD$_2$ levels in the plasma of MDD patients could not possibly represent the change in PGD$_2$ levels in the brain. To explore the change in PGD$_2$ levels in the brain, we applied a well-established animal model of depression by exposing mice to CUMS (Willner, 2005). Because the reduction of plasma PGD$_2$ concentration in MDD patients occurs regardless of gender, we used only male mice in this study. After exposure to CUMS for 5 weeks, mice exhibited depression-like behaviors, including reduced sucrose preference representative of anhedonia (Willner et al., 1987) and extended immobility time indicative of behavioral despair (Cryan et al., 2002). We extracted PGD$_2$ from the mouse brains and performed PGD$_2$ quantitation. Our inference that exposure to CUMS inhibited PGD$_2$ production was consistent with the decrease in plasma PGD$_2$ levels found in MDD patients. It would be valuable if the plasma PGD$_2$ levels of the CUMS mice could be measured. However, because the plasma PGD$_2$ levels of mice under normal conditions were extremely low and because of the limits of our current technology, we failed to detect the PGD$_2$ levels in mice plasma.

Previous studies have demonstrated that the physiological and pharmacological actions of PGD$_2$ in the CNS seem to be associated with the symptoms exhibited by MDD patients (Ohinata et al., 2008; Urade and Hayaishi, 2011). We tested whether the application of SeCl$_4$ can induce depression-like behaviors. Indeed, the administration of SeCl$_4$ caused the phenotype of behavioral despair in mice, as represented by a remarkably longer immobility time in the FST. The administration of SeCl$_4$ also inhibited spontaneous locomotor activity in mice. To exclude the possibility that the hypolocomotion was caused by muscle dysfunction, which in turn might affect the immobility in the FST, the grip strength of mice injected with SeCl$_4$ was tested. As a result, the administration of SeCl$_4$ showed no influence on the performance of mice in the GST. This finding revealed that SeCl$_4$-induced increased immobility in the FST was primarily an indication of behavioral despair. This conclusion was further confirmed by the fact that pretreatment with the antidepressant imipramine completely reversed the increases in immobility time induced by SeCl$_4$ in the FST. PGD$_2$ inhibition showed no influence on the time of mice spent in the central zone in the OFT might indicate that there was no relationship between PGD$_2$ reduction and anxious behavior, which was in line with the result that patients with anxious and nonanxious depression had no difference in plasma PGD$_2$ levels. Behavioral despair was suggested to be associated with glutamate release and NMDA receptor-mediated transmission in the CA3 region of hippocampus (Wang et al., 2015), while anhedonia was mainly regulated by medial prefrontal cortex-controlled interactions between the dopaminergic midbrain and the striatum (Ferenczi et al., 2016). Future studies exploring the underlying mechanism and pathway involved in the effect of PGD$_2$ in depression may help to explain the different influences of inhibited PGD$_2$ production in the SPT and FST.

Several mechanisms may account for the depression-like behaviors caused by inhibited PGD$_2$ production. For instance, X. Liang et al. (Liang et al., 2005) reported that PGD$_2$ induced cAMP production and mediated neuronal protection via the DP1 receptor; however, cAMP signaling was found to be decreased in the brains of unmedicated depressed patients (Fujita et al., 2016). Thus, by influencing cAMP signaling, resultant deficiencies in PGD$_2$ may ultimately lead to depressive symptoms. Besides, accumulating evidence indicates
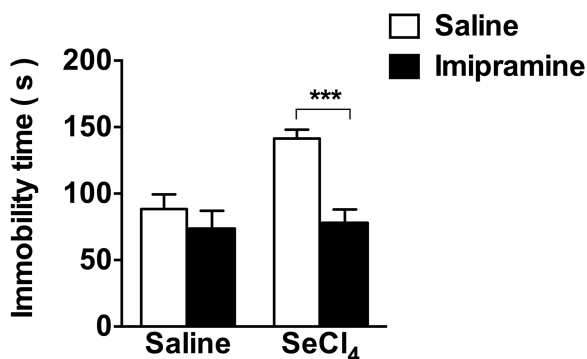
Figure 4. Reversal of 2.5 mg/kg SeCl$_4$-induced behavioral despair by imipramine (20 mg/kg) in the forced swim test (FST). Two-way ANOVA followed by simple main effects analysis (n = 7/group). Data are mean ± SEM. ***P < .001.

that inflammation may participate in the development of MDD. Proinflammatory cytokines such as interleukin-1, tumor necrosis factor-alpha, and interferon–gamma have been associated with depressive behaviors in mice (Asnis et al., 2003; Goshen et al., 2008; Kaster et al., 2012). Recent evidence reveals that PGD$_2$ is an antiinflammatory regulator and participates in the resolution of inflammation (Gilroy et al., 1999; Yoon et al., 2008; Kong et al., 2016). As a result, deficiency in PGD$_2$ may lead to a failure of inflammatory resolution, which in turn accelerates depression. However, the exact mechanism remains to be clarified.

To the best of our knowledge, this is the first study to explore PGD$_2$ levels in the peripheral blood of MDD patients. It is also the first investigation of PGD$_2$ levels in the animal depression model. The results should be validated in a larger sample and repeated in additional animal models of depression. In addition to pharmacological inhibitors, a better method for suppressing PGD$_2$ production is also needed to clarify the role of PGD$_2$ in MDD. Moreover, samples from the CNS of MDD patients, such as cerebrospinal fluid, should be used to investigate changes in PGD$_2$ levels.

In summary, the present study has clearly demonstrated that PGD$_2$ levels are decreased in the plasma of MDD patients and in the brains of depressive mice. The inhibition of PGD$_2$ production can lead to depression-like behaviors in mice. Further understanding of the molecular mechanisms of PGD$_2$ involvement in MDD may benefit the development of preventive and therapeutic targets for MDD in the future.

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Statement of Interest

None.

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