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Chronic treatment with celecoxib reverses chronic unpredictable stress-induced depressive-like behavior via reducing cyclooxygenase-2 expression in rat brain

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ABSTRACT

Recent clinical trails reported that adjunctive cyclooxygenase (COX)-2 inhibition with celecoxib is beneficial in treating depression. However, another clinical study showed celecoxib did not have inhibitory effect of COX-2 in human brain when given at a therapeutic dose. Therefore, whether celecoxib is exerting its influence through COX inhibition or by some other mechanism remains unclear. The present study further investigated the effect of celecoxib on COX-2 expression, prostaglandin E_2 (PGE_2, a major COX-2-mediated inflammatory mediator) concentration and the depressive-like behaviors in rats. Celecoxib was administrated by oral gavage to naive rats (16 mg/kg) or stressed rats (2, 8, 16 mg/kg, respectively) for 21 days, or to stressed rats for a single dose (16 mg/kg). The results showed that 21 days chronic unpredictable stress induced depressive-like behaviors and increased the COX-2 expression and PGE_2 concentration in rat brain. Chronic treatments with celecoxib alleviated the depressive-like behavior and reversed the levels of COX-2 expression and PGE_2 concentration in stressed rat in a dose-dependent manner. Celecoxib also improved the emotional state and decreased COX-2 expression and PGE_2 concentration in naive rats. In addition, a single dose of celecoxib treatment reversed COX-2 expression and PGE_2 concentration, but didn't alter the depressive-like behavior in stressed rat. These results suggest that COX-2 enzyme might play a key role in pathophysiology of depression. Furthermore, these data indicate that chronic celecoxib treatment reverse chronic unpredictable stress-induced depressive-like behavior might via reducing COX-2 enzyme in brain, and the selective COX-2 inhibitors could be developed as potential remedies for the management of depression.

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1. Introduction

Depression is a common and debilitating disorder for which current treatments are inadequate. The pathogenesis of depression is not well understood. Current antidepressants, which target monoamines, only produce remission in 30% of patients (McNally et al., 2008). Part of the problem lies in the fact that the pathophysiology of depression has not been elucidated, and treatments are based on empirical data, not mechanisms of action. Increasing amounts of data suggest that inflammatory responses have an important role in the pathophysiology of depression. Depressed patients have been found to have higher levels of proinflammatory cytokines, acute phase proteins, chemokines and cellular adhesion molecules (Dantzer et al., 2008; Raison et al., 2006).

Arachidonic acid derivatives such as prostaglandins play an important role in the inflammatory response (Pace et al., 2007). Cyclooxygenase (COX) is a rate-limiting enzyme in the metabolism of arachidonic acid to prostaglandins. COX exists mainly in two distinct isoforms (COX-1 and COX-2). The importance of COX-2 in depressive pathology is highlighted by recent findings demonstrating that the inflammatory enzyme mediated many of the central effects of psychologically relevant stressors (Madrigal et al., 2003). Studies indicated that 2–6 h of immobilization stress caused an enhancement of COX-2 protein expression in cortex and hippocampus (Lukiw and Bazan, 1997; Madrigal et al., 2002, 2003).Recently, hippocampal upregulation of the COX-2 mRNA has been demonstrated in a rat model of depression (Cassano et al., 2006).

Clinical trails reported that adjunctive COX-2 inhibition with celecoxib is beneficial in treating depression (Muller et al., 2006; Nery et al., 2008). In addition, chronic administration of lamotrigine (a novel antimanic drug) can decrease brain COX-2 expression (Lee et al., 2008), which may account in part for their efficacy in depression. However, Dembo et al. (2005) shown that celecoxib does not reach COX-2 inhibitory levels in human brain when given at a therapeutic dose. Therefore, whether celecoxib is exerting its influence through COX inhibition or some other mechanism remains to be determined.

Celecoxib have shown to improve the behavioral and immune changes in the olfactory bulbectomised rat model (Myint et al., 2007). As chronic unpredictable stress acts as a predisposing and participating

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factor in the onset of depression in humans and chronic unpredictable stress paradigm produces behavioral deficits thought to model aspects of depression (Katz, 1982; Rygula et al., 2005; Willner, 2005), whether COX-2 expression is upregulated in chronic unpredictable stress rat or whether celecoxib has the potential antidepressant effect in chronic unpredictable stress rat remains unknown.

Based on the above, we examined the effects of celecoxib in rats exposed to chronic unpredictable stress in the present study. Celecoxib was administered to naive or stressed rats for 21 days, or to stressed rats for a single dose. The antidepressant-like potential of celecoxib was then evaluated by using behavioral alterations caused by chronic unpredictable stress. Furthermore, levels of COX-2 protein and COX-2 mRNA and prostaglandin E_2 (PGE_2, a major COX-2-mediated inflammatory mediator) concentration in rat brain were also measured.

2. Material and methods

2.1. Animals

Male Sprague-Dawley rats (Beijing Weitong Lihua Research Center for Experimental Animals), weighing between 200 and 220 g, were used in the experiments. Rats were kept on a 12:12 h in the light: dark cycle (lights on at 7:00 AM, lights off at 7:00 PM) in individual home cages with food and water available ad libitum except as described in stress. All experiments conformed to the guidelines of the P. R. China legislations on the ethical use and care of laboratory animals. All efforts were made to minimize animal suffering and the number of animals needed to produce reliable data.

2.2. Groups and treatment

To observe the effects of celecoxib on depression model rats, 70 rats (10 in each group) were randomly divided into seven groups: The first group (control) received once-daily oral gavage (p.o.) administration of distilled water for 21 days. The second group (chronic unpredictable stress) received once-daily p.o. administration of distilled water for 21 days. The third group (chronic celecoxib, 16 mg/kg) received once-daily p.o. administration of celecoxib for 21 days. The fourth group (chronic unpredictable stress and chronic celecoxib, 16 mg/kg) received once-daily p.o. administration of celecoxib for 21 days. The fifth group (chronic unpredictable stress and chronic celecoxib, 8 mg/kg) received once-daily p.o. administration of celecoxib for 21 days. The sixth group (chronic unpredictable stress and chronic celecoxib, 2 mg/kg) received once-daily p.o. administration of celecoxib for 21 days. The seventh group (chronic unpredictable stress and a single dose celecoxib, 16 mg/kg) received once-daily p.o. administration of distilled water for 20 days followed by a single celecoxib p.o. aministration at day 21. Celecoxib (marketed as Celebrex, Pfizer Inc., USA) was diluted in distilled water and orally given one hour before the stress exposure.

2.3. Chronic unpredictable stress procedure

The chronically stress procedure was modified from procedures used by Heine et al. (2004). Briefly, rats were exposed to different stressors daily for 21 days as follows. day1: cold immobilization for 1 h at 4 °C, forced swim for 30 min at 25 °C; day 2: immobilization for 1 h, crowding for 23 h; day 3: forced cold swim stress for 5 min at 10 °C, isolation for 23 h; day 4: immobilization for 1 h, vibration for 1 h; day 5: forced swim stress for 30 min at 25 °C, cold immobilization for 1 h at 4 °C; day 6: forced cold swim stress for 5 min at 10 °C, crowding for 23 h; day 7: vibration for 1 h, isolation for 23 h. This schedule was repeated twice for a total of 21 days. Prior to the study, certain criteria were set for excluding animal on weight loss, or the possible occurrence of wounds. Rats were acclimated to 3 min of handling once a day for 7 consecutive days before being used in experiment and were weighed on the 1st and 7th day of handling.

2.4. Body weight measurement

Rats were weighed on day 1 and 21 of the chronic unpredictable stress experiment.

2.5. Sucrose preference test

Sucrose preference tests were used to operationally define anhedonia. Specifically, anhedonia was defined as a reduction in sucrose intake and sucrose preference relative to the intake and preference of the control group. A sucrose preference test consisted of first removing the food and water from each rat's cage for a period of 20 h. Water and 1% sucrose were then placed on the cages in preweighed glass bottles, and animals were allowed to consume the fluids freely for a period of 1 h. Two baseline preference tests were performed, separated by at least 5 days, and the results were averaged. A preference test was also conducted following the 21 days chronic unpredictable stress period. On the last-stressed day, rats were deprived of water and food for 20 h, then from the next day on, rats were given a 1 h window sucrose preference test (24 h after the last drug treatment). Sucrose and water consumption (ml) was measured and the sucrose preference was calculated as the sucrose preference (%) = sucrose consumption / (sucrose consumption + water consumption).

2.6. Open field exploratory behavior test

Open field test was used to study the exploratory and anxiety behavior of rats and was performed after the sucrose preference test. The open field apparatus consisted of a square arena 60 × 60 cm with 40 cm high wall. The entire apparatus was painted black except for 6 mm white lines that divided the floor into 16 equal size squares. The squares were subdivided into peripheral and central sector, where the central sector included the 4 central squares (2 × 2) and the peripheral sector contained the squares close to the wall. The apparatus was illuminated with a low intensity diffuse light (45 W) situated 45 cm above the floor level. Entire room, except the open field was kept dark during the experiment. Each animal was placed in the central square and observed for 5 min by a video camera and taped for further analysis. Motility was scored when an animal crossed a sector border with both its hind-limbs. The following behaviors were scored by an observer who was blind to the drug treatment. Central ambulation: number of central squares crossed; Total ambulation: the overall number of peripheral and central square crossed; rearing: number of times the animal stood on its hind limbs; grooming: number of times the animal made these responses viz. grooming of the face, licking/cleaning and scratching the various parts of the body. Immobility period: the time spent immobile. Anxiety-related behavior was measured by the percentage of central ambulation and calculated as the percentage of central ambulation (%) = central ambulation / total ambulation. Between tests, the apparatus was cleaned with 5% alcohol.

2.7. Western blot analysis

Following the chronic unpredictable stress period and post-chronic unpredictable stress sucrose preference test, rats were sacrificed via decapitation 24 h after last stress. Then the brain was dissected and put into chilled tubes treated with an enzyme inhibitor. Brain tissue was homogenized and Western blot analysis carried out as previously reported (Guo et al., 2006a), using primary antibodies for COX-2 (1:2000, Santa Cruz Biotechnology) and β-actin (1:10000, Santa Cruz Biotechnology). A secondary antibody conjugated with

horseradish peroxidase (HRP, 1:5000, Bio-Rad) was used. Immunoblots were visualized on X-ray film by chemiluminescence reaction (Pierce), and image analysis was performed on optical density-calibrated images by AlphaEase Stand Alone software (Alpha Innotech).

2.8. Total RNA isolation and real time RT-PCR

COX-2 mRNA in brain was measured by real-time reverse transcription PCR (RT-PCR) as previously described (Guo et al., 2006b). Briefly, the total RNA from brain was obtained by TRIzol method. RNA was reverse transcribed to cDNA using the Taqman® Reverse Transcription Reagents (Applied Biosystems, USA). Real-time quantitative PCR analyses for COX-2 and GAPDH were performed in 96-well plates using the ABI PRISM 7700 Sequence Detection System instrument and software (PE Applied Biosystems). PCR were performed with the SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacturer's protocol, using the following oligonucleotide primers: COX-2-forward 5′-TTTGTTGAGTCATTCACCA-GACAGAT-3′ and reverse 5′-ACGATGTGTAAGGTTTCAGGGAGAAG-3′ (169 bp); GAPDH-forward 5′-TGAACGGGAAGCTCACTGG-3′and reverse 5′-GAGCTTCACAAAGTTGTCATTGAG-3'(260 bp). The basic protocol for real-time PCR was an initial incubation at 95 °C for 5 min, followed by 45 cycles of 95 °C for 30 s, 56 °C for 30 s, 72 °C for 1 min and finally cooling to 40 °C. All samples were run in triplicate, the relative expression values were normalized with GAPDH value.

Plasmids containing cDNA was used as standard in quantifying the PCR results. The interest cDNA was amplified by RT-PCR using the same premiers as for real-time RT-PCR. The PCR products were cloned into pGEM-T easy vector (Invitrogen) and confirmed by sequencing. The purified recombinant plasmid DNA was quantified by UV spectrophotometer and then serially diluted in double-distilled water as standard for numerical quantification. The standard curve prepared for COX-2 and GAPDH was used as housekeeping gene. The PCR products were sequenced to verify the analytical specificity. Melting curve was analyzed after PCR amplification.

2.9. Measurement of PGE2 concentration

Levels of PGE_2 were determined in microwaved brain extracts. Brains were weighed, then extracted in 18 volumes of hexane: 2-propanol (3:2, by volume) using a glass Tenbroeck homogenizer. The prostaglandins were purified from the lipid extract using a C_{18} Sep-Pak cartridge (Waters) by the method of Powell (1985). The concentration of PGE_2 was determined using an enzyme-linked immunosorbent assay (ELISA) (Invitrogen) and were normalized with a protein assay kit.

2.10. Statistical analysis

Data are expressed as means ± SEM. Differences among groups were examined using Kruskal–Wallis test, followed by Dunn's multiple comparisons test. Data were correlated by nonparametric Spearman's rank method. P value <0.05 was considered statistically significant.

3. Results

3.1. Body weight measurement

At the 1st day of the chronic unpredictable stress period, rats from different groups showed no significant difference in body weight (Kruskal–Wallis $H=1.89$, $P>0.05$). However, significantly difference was observed among groups following 21 days of chronic unpredictable stress (Kruskal–Wallis $H=23.7$, $P<0.01$). The chronic unpredictable stress group gained body weight more slowly than control, as shown in Fig. 1. It can be seen that there was a significant effect of stress treatment ($P<0.01$). Average of body weight was 332.5 g at the 21 day in the control, compared with 268.8 g in the chronic unpredictable stress group. Chronic treatments with celecoxib had no intrinsic effect but significantly increased the body weight of rats compared with chronic unpredictable stress group in a dose-dependent manner. A single dose of celecoxib treatment had not effect on chronic unpredictable stress-induced decrease in body weight.

3.2. Sucrose preference tests

Before the chronic unpredictable stress period, rats from different groups showed no significant difference in sucrose solution intake and sucrose preference (data not shown). Fig. 2A and B displays the liquid intake and preference for sucrose in seven groups following 21 days of chronic unpredictable stress. Kruskal–Wallis test indicated that both the sucrose solution intake and the sucrose preference significantly differed among groups ($H=23.42$, $P<0.01$, and $H=25.05$, $P<0.01$, respectively). The 21 days exposure to chronic unpredictable stress induced a reduction in sucrose preference and sucrose solution intake, which is indicative of operationally defined anhedonia. The sucrose solution intake and sucrose preference were significantly reduced in the chronic unpredictable stress group relative to the control group ($P<0.01$). Mild increases in sucrose solution intake and sucrose preference were observed in chronic celecoxib treatment group compared with control group. Chronic treatment with celecoxib significantly suppressed the chronic unpredictable stress-induced decrease in sucrose preference and sucrose solution intake in a dose-dependent manner. A single dose of celecoxib treatment didn't alter the stress-induced sucrose preference and sucrose solution intake.

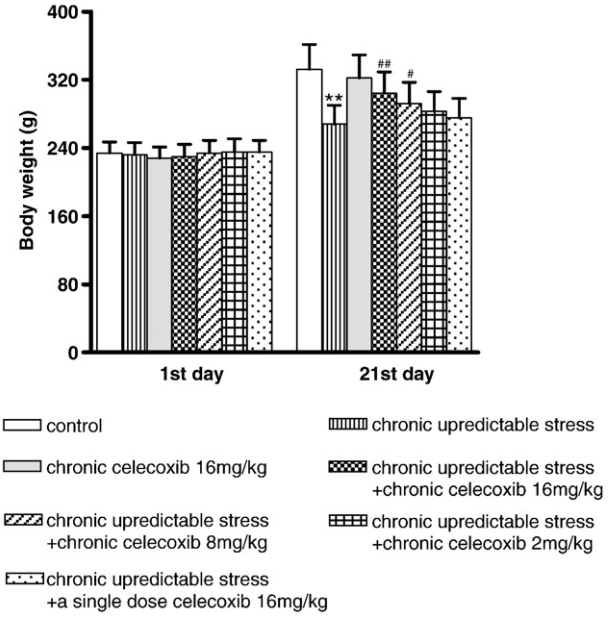

Fig. 1. Effect of celecoxib on body weight on day 1 and 21 of chronic unpredictable stress. At the 1st day of the chronic unpredictable stress period, rats from different groups showed no significant difference in body weight. However, significantly difference was observed among groups following 21 days of chronic unpredictable stress. The 21 days exposure to chronic unpredictable stress significantly decreased the body weight in comparison to the control group ($P<0.01$). Chronic treatments with celecoxib had no intrinsic effect but significantly augmented the body weight of rats compared with chronic unpredictable stress group in a dose-dependent manner. A single dose of celecoxib treatment had not effect on chronic unpredictable stress-induced decrease in body weight. $^{**}P<0.01$, as compared to the control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, as compared to the chronic unpredictable stress group.

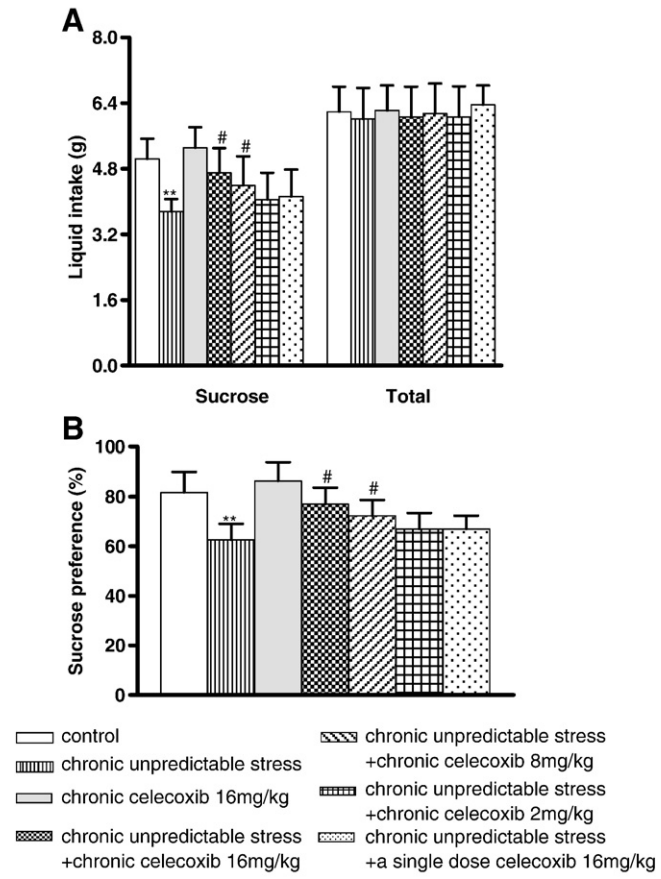

Fig. 2. Effect of celecoxib on liquid intake and sucrose preference following 21 days of chronic unpredictable stress. Sucrose solution intake and sucrose preference was reduced in the chronic unpredictable stress group compared with control group ($P<0.01$). Mild increases in sucrose solution intake and sucrose preference were observed in control plus chronic celecoxib treatment group. Chronic treatment with celecoxib significantly augmented the decrease in sucrose solution intake and sucrose preference in a dose-dependent manner. A single dose of celecoxib treatment did not alter stress-induced behavior alterations. The total liquid intake did not differ among groups. $^{**}P<0.01$, as compared to the control group; $^{\#}P<0.05$, as compared to the chronic unpredictable stress group.

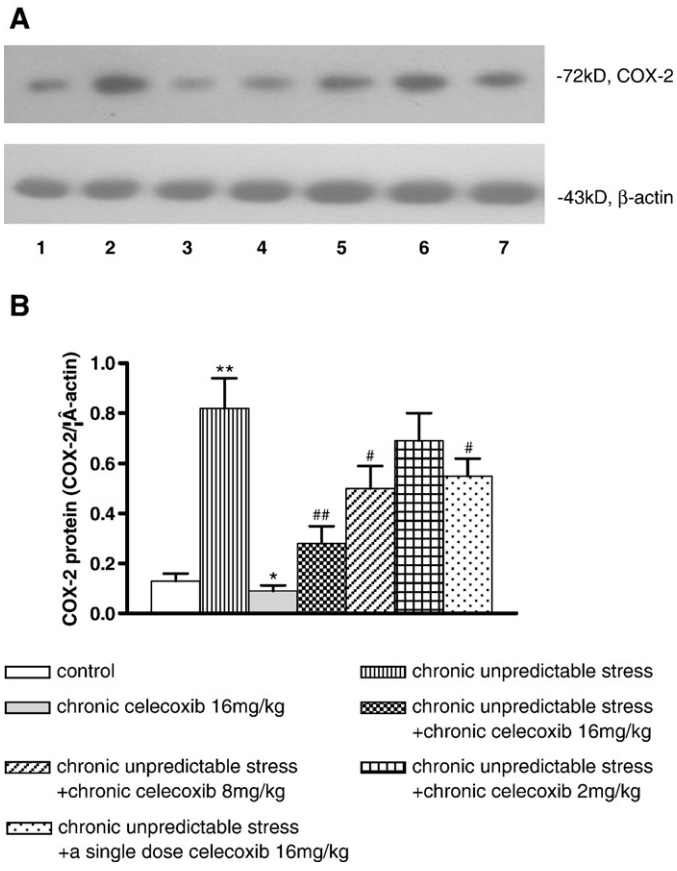

Fig. 3. Effect of celecoxib on brain COX-2 protein level following 21 days of chronic unpredictable stress. A. Representative immunoblots of COX-2 and β-actin. 1, control; 2, chronic unpredictable stress; 3, chronic celecoxib(16 mg/kg); 4, chronic unpredictable stress plus chronic celecoxib(16 mg/kg); 5, chronic unpredictable stress plus chronic celecoxib(8 mg/kg); 6, chronic unpredictable stress plus chronic celecoxib(2 mg/kg); 7, chronic unpredictable stress plus a single dose of celecoxib (16 mg/kg). B. Relative optical density (OD) of COX-2 to β-actin. $^{*}P<0.05$, $^{**}P<0.01$, as compared to the control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, as compared to the chronic unpredictable stress group.

The total liquid intake did not differ among groups (Kruskal–Wallis $H=1.01$, $P>0.05$).

3.3. Open field exploratory behavior test

Kruskal–Wallis test indicated significant differences among groups in the total ambulation ($H=22.54$, $P<0.01$), percentage of center ambulation ($H=24.25$, $P<0.01$), rearing ($H=31.89$, $P<0.01$), grooming ($H=42.2$, $P<0.01$) and immobility period ($H=46.17$, $P<0.01$). Chronic unpredictable stress rats exhibited decreased total ambulation, percentage of center ambulation, rearing, increased grooming and immobility period in comparison to control rats ($P<0.01$). Chronic treatment with celecoxib *per se* significantly increased the percentage of center ambulation ($P<0.05$), but didn't affect other stress-induced behavior alterations, indicating that chronic celecoxib treatment attenuated the anxious behavior. Chronic treatments with celecoxib also significantly reversed the stress-induced behavioral alterations in a dose-dependent manner, as observed by increased total ambulation, percentage of central ambulation, rearing, decreased grooming and immobility period as compared to the chronic unpredictable stress group. A single dose of celecoxib treatment didn't affect the stress-induced behavior alterations in open field test (Table 1).

Table 1
Effect of celecoxib on open field exploratory test in chronic unpredictable stress rats.

Groups	Total ambulation	Central ambulation (%)	Rearing	Grooming	Immobility period (s)
Control	31.7 ± 2.3	29.6 ± 2.1	23.6 ± 3.1	4.6 ± 0.7	65.5 ± 6.8
Chronic unpredictable stress	20.3 ± 3.1^{a}	20.2 ± 1.5^{a}	10.1 ± 1.0^{a}	11.04 ± 1.2^{a}	200.4 ± 23.9^{a}
Chronic celecoxib (16 mg/kg)	32.4 ± 2.5	34.4 ± 1.9^{b}	24.6 ± 2.8	4.3 ± 0.8	59.9 ± 6.7
Chronic unpredictable stress + chronic celecoxib (16 mg/kg)	28.7 ± 3.1^{c}	27.8 ± 2.2^{c}	20.4 ± 2.4^{c}	6.6 ± 1.1^{c}	76.1 ± 9.4^{c}
Chronic unpredictable stress + chronic celecoxib (8 mg/kg)	26.1 ± 2.4	26.5 ± 2.7^{d}	18.8 ± 1.9^{c}	7.2 ± 0.9^{d}	93.8 ± 13.5^{c}
Chronic unpredictable stress + chronic celecoxib (2 mg/kg)	22.0 ± 1.8	21.9 ± 2.3	13.7 ± 1.8	9.8 ± 0.9	140.7 ± 19.7^{d}
Chronic unpredictable stress + a single dose celecoxib (16 mg/kg)	21.4 ± 2.5	21.3 ± 1.9	11.7 ± 1.5	9.9 ± 1.1	189.0 ± 10.2

Data are expressed as mean ± SEM of 10 rats per group.^{a,b}Significantly different from control group value ($^{a}P<0.05$; $^{b}<0.01$). $^{c,\ d}$Significantly different from chronic unpredictable stress group value ($^{c}P<0.05$; $^{d}P<0.01$).

3.4. COX-2 protein level

In Western blots, the COX-2 antibody detected a prominent band at about 72 kDa (Fig. 3A). Kruskal–Wallis test indicated that COX-2 protein level significantly differed among groups ($H=54.55$, $P<0.01$). In the chronic unpredictable stress group, the COX-2 protein level was increased by 5.7-fold ($P<0.01$) compared to the control level (Fig. 3B). A significant decrease in COX-2 protein level was demonstrated in chronic celecoxib treatment group compared with control group ($P<0.05$). Chronic treatments with celecoxib significantly inhibited the chronic unpredictable stress-induced COX-2 protein level in a dose-dependent manner. A single dose of celecoxib treatment also markedly reduced the chronic unpredictable stress-induced COX-2 protein ($P<0.05$).

3.5. COX-2 gene expression

Real-time quantitative PCR was used to measure the mRNA level of COX-2 in rat brain. The standard curve was draw for COX-2 and GAPDH gene (for example, COX-2 in Fig. 4A and B). Melting curve analysis confirmed that there was no primer dimer in the PCR products (Fig. 4C). For each primer set, non-specific amplification was seen after agarose gels electrophoresis and ethidium bromide staining (Fig. 4D). Kruskal–Wallis test indicated that COX-2 mRNA expression significantly differed among groups ($H=50.13$, $P<0.01$). COX-2 mRNA expression was upregulated in chronic unpredictable stress group compared with the control group ($P<0.01$). A mild decrease in COX-2 mRNA expression was observed in chronic celecoxib treatment group compared with control group (Fig. 4E). Chronic treatments with celecoxib significantly inhibited the chronic unpredictable stress-induced COX-2 mRNA expression in a dose-dependent manner. A single dose of celecoxib treatment also markedly reduced the stress-induced COX-2 mRNA expression ($P<0.05$).

3.6. PGE_2 concentration

To see if the observed reduction of COX-2 protein was accompanied by a decrease in PGE_2, an arachidonic acid metabolite produced by COX-2, we measured PGE_2 in rat brain. Kruskal–Wallis test indicated that PGE_2 concentration significantly differed among groups ($H=42.98$, $P<0.01$). The brain PGE_2 concentration in chronic unpredictable stress group was higher than these in the control level (84.9 ± 7.2 pg/mg versus 38.6 ± 4.4 pg/mg, respectively; $P<0.01$). A significant decrease in PGE_2 concentration was observed in chronic celecoxib treatment group compared with control group ($P<0.05$). Chronic treatments with celecoxib significantly inhibited the chronic unpredictable stress-induced PGE_2 concentration in a dose-dependent manner (Fig. 5). A single dose of celecoxib treatment also significantly reduced the stress-induced PGE_2 concentration ($P<0.05$).

3.7. Correlations

According to Spearman's nonparametric rank correlation method, data analysis revealed that chronic celecoxib treatments-mediated reduction of chronic unpredictable stress-induced COX-2 protein correlated with an increase in body weight gain ($r=-0.92$, $P<0.05$), or with an increase in sucrose preference ($r=-0.93$, $P<0.05$).

4. Discussion

The chronic mild stress model of depression as described by Willner et al. (1987) is accepted as a valuable method for inducing experimental depression in rats. In the model, various stressors are applied in unpredictable order, simulating conditions in the natural environment for the major purpose of inducing anhedonia-like behavioral change, i.e. inability to experience pleasure, which is the

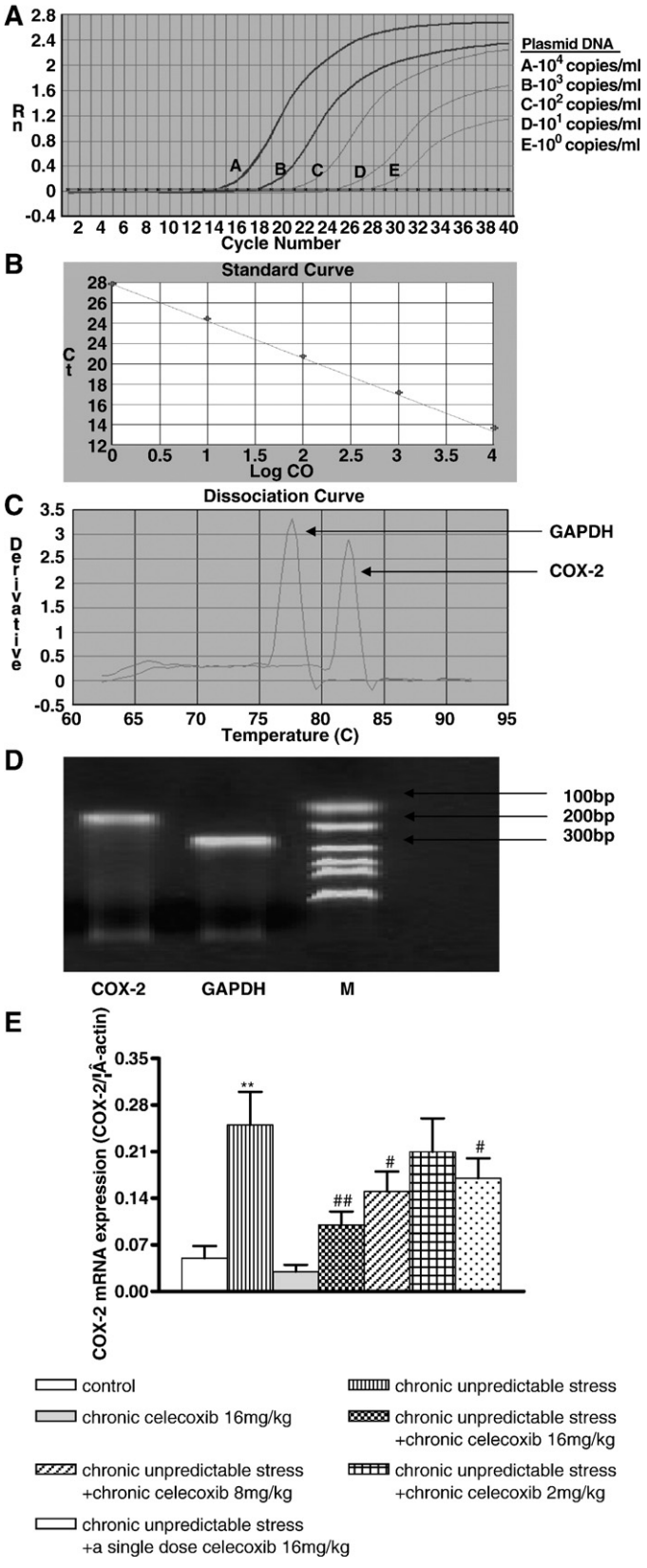

Fig. 4. Real-time quantiative PCR analysis. A and B. Linear stand curve from plasmid DNA concentrations of COX-2. C. Melting curves of the amplification products from COX-2 and GAPDH. D. Gel electrophoretic analysis. Cytokines mRNA-specific DNA bands were identified by analyzing the real-time PCR products on 1.5% agarose gel. M, Molecular marker; predicted lengths of the PCR products were 260 bp (GAPDH), 169 bp (COX-2). E. Effect of celecoxib on brain COX-2 mRNA expression following 21 days of chronic unpredictable stress. $^{**}P<0.01$, as compared to the control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, as compared to the chronic unpredictable stress group.

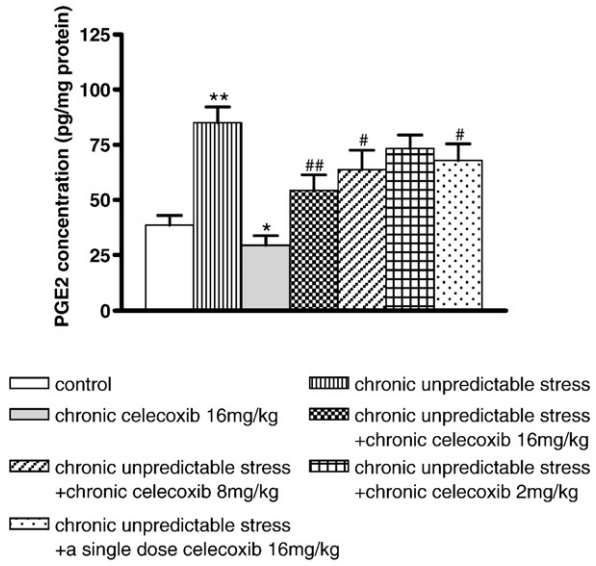

Fig. 5. Effect of celecoxib on brain PGE_2 concentration following 21 days of chronic unpredictable stress. The brain PGE_2 concentration in chronic unpredictable stress group was significantly higher than these in the control level ($P<0.01$). A significant decrease in PGE_2 concentration was observed in chronic celecoxib treatment group compared with control group ($P<0.05$). Chronic treatments with celecoxib significantly reduced the stress-induced PGE_2 concentration in a dose-dependent manner. A single dose of celecoxib treatment also significantly reduced the stress-induced PGE_2 concentration ($P<0.05$). $^{*}P<0.05$, $^{**}P<0.01$, as compared to the control group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, as compared to the chronic unpredictable stress group.

core symptom of human major depression (Willner, 1997). Anhedonia has been defined as decreased responsiveness to rewards (Anisman and Matheson, 2005; McArthur and Borsini, 2006), which is measured originally by decreased intake of a palatable sweet solution (Willner et al., 1987). While Matthews et al. (1995) showed stress-induced decrease in body weight, which is another important symptom of depression, reduce locomotor activity of rats in open field test (D'Aquila et al., 2000), which may mimic some aspects of human psychomotor retardation (Willner et al., 1987), an accompanying symptom of major depression in humans (Anisman and Matheson, 2005). In this experimental conditions, there has been a significantly reduction of sucrose preference in chronic unpredictable stress group compared with the control, that was reduced to approximately 30% at day 21 after the beginning of stress exposure. These results demonstrate an operational change in reward sensitivity associated with chronic unpredictable stress. Chronic unpredictable stress rats also exhibited decreased body weight and anxious behavior which is porved by decreased ambulation, rearing, increased grooming and immobility period in comparison to control rats.

Arachidonic acid is converted via the COX pathway to prostaglandin G_2 (PGG_2), which in turn is rapidly converted to prostaglandin H_2 (PGH_2) and then to prostaglandins, prostacyclins, and thromboxanes by tissue-specific terminal synthases (Herschman, 1996). These eicosanoids regulate many physiologic functions. There is limited evidence to date to link abnormal arachidonic acid signaling to depression. It has been suggested that stimulation of prostaglandin synthesis by prolactin or other hormones can contribute to mood disorders (Horrobin et al., 1978). Furthermore, PGE_2 is reported to be increased in the plasma and cerebrospinal fluid of depressed patients (Lieb et al., 1983), and recent investigation into the pathophysiology of psychiatric illness implicated the arachidonic acid cascade (Rapoport and Bosetti, 2002), This study shows that exposure to chronic unpredictable stress significantly increased protein and mRNA levels of COX-2, and PGE_2 concentration in rat brain. Thus, chronic unpredictable stress had an upregulatory effect on one aspect of the brain arachidonic acid cascade. Of particular interest are the data derived from studies of anti-mania drugs. In rat, chronic administration of lithium was found to decrease turnover of COX-2 protein expression, COX-2 activity, and PGE_2 in brain, whereas COX-1 protein was not altered (Bosetti et al., 2002). Similar to lithium, COX-2 mRNA and protein in brain was reduced by chronic lamotrigine administration (Lee et al., 2008). Together with our findings, these studies lend further support to hypotheses that arachidonic acid metabolism and inflammatory response may play important roles in the pathophysiology of depression.

To elucidate the mechanisms underlying the antidepressant effects of celecoxib, we observed the changes of body weight, liquid intake, sucrose preference, behavioral alteration, COX-2 expression and PGE_2 concentration in chronic unpredictable stress plus chronic celecoxib treatment rat. The results revealed that chronic celecoxib treatment could increase the stress-induced decrease in body weight, sucrose solution intake, sucrose preference, and reversed the stress induced behavioral alteration in a dose-dependent manner. We also found chronic celecoxib treatment could suppress the stress-induced elevation in levels of COX-2 protein and COX-2 mRNA and PGE_2 concentration in rat brain in a dose-dependent manner. According to Spearman's nonparametric rank correlation method, data analysis revealed that chronic celecoxib treatments-mediated reduction of chronic unpredictable stress-induced COX-2 protein correlated with an increase in body weight gain or sucrose preference. Green tea polyphenols have been found to reverse lipopolysaccharide (LPS)-induced immobility in mice possible by COX-2 mechanism (Singal et al., 2004). All together, our data therefore suggest that chronic celecoxib treatment reverses chronic unpredictable stress-induced depressive-like behavior might via reducing COX-2 enzyme in brain. Some anti-manic drugs also decreases brain COX-2 expression as mentioned above (Bosetti et al., 2002; Lee et al., 2008), and this decrease may be related to the clinical action of the drugs. However, Brunello et al. (2006) found that acetylsalicylic acid accelerated the onset of action of fluoxetine in chronic escape deficit model of depression but it was ineffective alone. This may be because celecoxib is a selective COX-2 inhibitors, and is a more potent compound centrally distributions and mainly expected to act centrally after oral administration (Okumura et al., 2006). We also observe the effect of chronic celecoxib treatment in naive rats. In comparison with control animals, chronic celecoxib treatment rats exhibited increased central locomotion, mildly increased sucrose solution intake and sucrose preference, which indicates that celecoxib improves the emotional state in the naive animals. Chronic celecoxib treatment also *per se* reduced levels of COX-2 protein and COX-2 mRNA and PGE_2 concentration in rat brain.

A previous clinical study shows that celecoxib does not reach COX-2 inhibitory levels in human brain when given at a therapeutic dose (Dembo et al., 2005). Here we also observed the effect of celecoxib in chronic unpredictable stress-induced rats after a single dose of 16 mg/kg administration. Since using allometric calculation, 16 mg/kg for rat correspond to 181 mg for a man with 70 kg, which is within the clinical dose of celecoxib but below the dose used in the antidepressant trials by Dembo et al. (2005). Differing with their results, the present study indicates that a single dose of celecoxib (16 mg/kg) treatment could reduce the stress-induced COX-2 protein and PGE_2 concentration in rat brain. Possible reasons for the discrepancy may that the blood brain barrier passage between rats and humans is different. In addition, a single dose of celecoxib treatment didn't alter the stress-induced behavior alterations. These results demonstrate that the levels of COX-2 mRNA expression and protein must be below a certain value to produce antidepressant-like activity. Therefore, repeated, but not a single dose, administration of celecoxib may offer a protective effect against stressed-induced depressive-like behavior.

There is a controversy regarding the effect of nonsteroidal antiinflammatory drugs (NSAIDs) on the expression of COX-2 mRNA and protein. NSAIDs has bee reported to inhibit COX-2 enzyme activity but not mRNA expression in human macrophages (Barrios-Rodiles et al., 1996). Celecoxib did not change COX-2 mRNA level in candesartan-treated rat kidney (Höcherl et al., 2001). On the contrary, in human endothelial cells stimulated with interleukin-1 it has been shown that aspirin and naproxen can inhibit the expression of COX-2 at the levels of transcription and translation (Wu et al., 1991; Zyglewska et al., 1992). Celecoxib has been shown to possess the antitumor activity on K562 leukemia cells via downregulation of COX-2 mRNA and protein expression (Zhang et al., 2006). In the present study, we found that celecoxib reduced the chronic unpredictable stress-induced COX-2 protein and mRNA levels in rat brain. Collectively, these results suggest that COX-2 gene expression is differentially regulated by NSAIDs in a cell or tissue specific manner.

In summary, we demonstrated that celecoxib reverses chronic unpredictable stress-induced depressive-like behavior after 21 days treatment, but not a single dose treatment, like many antidepressants tested to date (Song and Leonard, 2005).We further showed that celecoxib reverses chronic unpredictable stress-induced depressive-like behavior might via reducing COX-2 expression in rat brain. To our knowledge, this study is the first to report celecoxib decreases the expression of COX-2 protein and mRNA in chronic unpredictable stress-induced rat brain. Future studies are needed to examine the effect of celecoxib on COX-2 expression and PGE2 concentration in discrete brain areas associated with depression and reward (e.g. frontal cortex and n. accumbens). It is possible that COX-2 may play a key role in the pathophysiology of depression. Although care must be paid in extrapolating data from rodents to a complex human depression, if COX-2 and PGE_2 were found to be increased in postmortem brain or in cerebrospinal fluid from depression patients, COX-2 inhibitors could represent a new therapeutic approach for the treatment of this disease.

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