Brain-derived neurotrophic factor signalling mediates antidepressant effects of lamotrigine

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Abstract

The anticonvulsant drug lamotrigine has been shown to produce antidepressant effects in patients with bipolar disorder. To date, only a few preclinical studies have been conducted using lamotrigine treatment in the forced swim test (FST), an animal model of depression with low face validity. The underlying mechanisms by which lamotrigine works have not been well characterized either. This study extends earlier work on the role of brain-derived neurotrophic factor (BDNF) in regulating the antidepressant actions of lamotrigine. We showed that in rats subjected to chronic unpredictable stress, chronic administration of 30 mg/kg lamotrigine ameliorates behavioural deficits of stressed rats in both sucrose preference test (SPT) and novelty-suppressed feeding test (NSFT). In parallel, chronic lamotrigine treatment up-regulates frontal and hippocampal BDNF protein expression in both naive and stressed animals, and restores the stress-induced down-regulation of BDNF levels. In addition, inhibition of BDNF signalling by infusion of K252a, an inhibitor of the BDNF receptor TrkB, blocks the antidepressant effects of lamotrigine in SPT, NSFT and FST. Taken together, this study provides further evidence that BDNF is an essential mediator for the antidepressant effects of lamotrigine.

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Introduction

Bipolar disorder is a severe, debilitating and recurring psychiatric disease that affects up to 4% of the world’s population (Calabrese et al. 1999). Despite the availability of a variety of mood stabilizers, treatment of the depressive phase remains challenging because many conventional antidepressants precipitate the onset of the manic phase (Bourin & Prica, 2007). In search of solutions to this dilemma, an anticonvulsant lamotrigine has emerged as a promising candidate. Lamotrigine has demonstrated moderate antidepressant efficacy in the treatment of bipolar and major depressive disorders (Bowden et al. 1999; Calabrese et al. 1999; Goodwin et al. 2008; Vigo & Baldessarini, 2009) without significant side-effects, such as the precipitation of mania (Calabrese et al. 2001).

Rodent studies have generated inconsistent results on the antidepressant effects of lamotrigine when using a behavioural despair model of depression, namely the forced swim test (FST) (Bourin & Prica, 2007). The controversy can be partially explained by the drug dose because only a high (>10 mg/kg) (Bourin et al. 2005; Consoni et al. 2006; Li et al. 2010), but not a low (<5 mg/kg) (Ali et al. 2003) dose of lamotrigine significantly reduced immobility in FST. Duration of treatment also has different effects. We previously demonstrated that subchronic (30 mg/kg, 7 d), but not acute treatment of lamotrigine ameliorated the deficits in the number of failure escapes in the learned-helplessness (LH) paradigm (Li et al. 2010). However, FST and LH models are responsive to acute or subchronic antidepressant treatments, whereas in clinical studies therapeutic responses generally require chronic treatments (Schmidt & Duman, 2007).
Therefore, in the present study, we utilize two behavioural models which are responsive to chronic antidepressant treatments and thus have better face validities, namely the chronic unpredictable stress (CUS) model, which mimics the anhedonia symptoms of depression (Willner, 2005), as well as the novelty-suppressed feeding test (NSFT), which models anxiety-like behaviour (Santarelli et al. 2003). Using a combination of CUS and NSFT, we have evaluated more convincingly the behavioural actions of lamotrigine in rodent models of depression.

The molecular mechanisms underlying the antidepressant effects of lamotrigine also remain largely elusive. Potential targets include neurotrophic factors, such as brain-derived neurotrophic factor (BDNF). The neurotrophic hypothesis of depression suggests that a variety of antidepressants function by positively regulating neurotrophic factors (Duman, 2004). Previous studies in our, as well as other laboratories, have shown that subchronic or chronic treatment of lamotrigine increased BDNF levels in the frontal cortex and hippocampus (Chang et al. 2009; Li et al. 2010). However, a direct link between BDNF signalling and behavioural actions of lamotrigine has not been established. In the present study, we test this hypothesis directly by infusing an inhibitor of the BDNF receptor TrkB, and subjecting animals to the aforementioned behavioural tests. Our results demonstrate that BDNF is a critical mediator of the antidepressant effects of lamotrigine.

Methods

Animals

A total of 48 male Sprague–Dawley rats (Beijing Laboratory Animal Centre, China) weighing 175–200 g were housed in pairs and maintained under standard conditions on a 12-h light/dark cycle (lights on 07:00 hours) with food and water available ad libitum. Two experiments were performed in this study, with six rats used per group (n = 6). The first experiment had two variables: stress condition (CUS and non-stressed, see below) and drug treatment (lamotrigine and vehicle). The second experiment, in which all rats received surgery and were subjected to the CUS procedure, also had two variables: infusion treatment (K252a and DMSO) and drug treatment (lamotrigine and vehicle). Behavioural tests were performed during the light phase of light/dark cycle. The experiments were performed in accordance with the guidelines of the Beijing Laboratory Animal Centre and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23).

Drug treatment

Lamotrigine (Sigma, USA) was dissolved in 0.5% carboxymethylcellulose, 0.4% Tween-80, 0.9% benzylic acid and saline, which served as the control vehicle in all assays. For CUS/behaviour experiments, animals were randomly assigned into different drug treatment groups, receiving a daily intraperitoneal (i.p.) injection of vehicle or 30 mg/kg lamotrigine. The dose was based on previous studies (Bourin et al. 2005; Consoni et al. 2006; Li et al. 2010; Prica et al. 2008). Lamotrigine treatment was initiated 1 wk after CUS began and lasted for 14 d. The TrkB receptor antagonist, K252a (2 mM; Calbiochem, USA), or the vehicle (DMSO, Sigma) was delivered in a 1 μl volume at the rate of 0.25 μl intracerebroventricularly (i.c.v.) with a cannula (26GA, PlasticOne, USA) protruding 0.5 mm beyond the guide cannula (PlasticOne) on days 14, 16, 18, 20. The dose and time-course for K252a were based on previous studies (Shirayama et al. 2002; Warner-Schmidt & Duman, 2007; Greene et al. 2009).

Stereotaxic surgery

Six days before the initiation of CUS, rats were anaesthetized with Nembutal (55 mg/kg i.p.; Henry Schein, USA), and a single guide cannula was stereotaxically placed into the lateral ventricle (coordinates relative to bregma: −0.9 mm AP, −1.5 mm ML, and −3.3 mm DV from dura). The cannula was held in place with aluminium screws and dental cement. Post-operative care consisted of perisurgical administration of carprofen (5 mg/kg) and triple antibiotics.

CUS procedure

In CUS, animals were exposed to a random sequence of mild and unpredictable stressors (Willner, 2005). Our CUS procedure has been successfully used to produce depressive-like behavioural changes (Warner-Schmidt & Duman, 2007; Greene et al. 2009). A total of 10 different stressors were used (two stressors per day for 21 d, see Fig. 1). The stressors included rotation on a shaker, placement in a cold environment, lights off for 3 h (10:00 to 13:00 hours), lights on overnight, strobe light overnight, aversive odour, cage tilted at 45°, food and water deprivation, crowded housing and isolated housing. The same CUS procedure was used for lamotrigine experiments with or without surgery. Control animals were handled every other day and animal weights were monitored.
**Sucrose preference test (SPT)**

In the SPT, rats were first exposed to a palatable sucrose solution (1%; Sigma) for 48 h (days 20 and 21), and on day 22, rats were deprived of water for 4 h, and were then exposed to two identical bottles for 1 h. One was filled with sucrose solution and the other with water. This procedure was adapted from previous studies (Greene et al. 2009). Sucrose and water consumption was determined by measuring the change in the liquid volume. Sucrose preference was defined as the ratio of the sucrose vs. the total consumption (sucrose and water) during the 1-h exposure.

**Novelty-suppressed feeding test (NSFT)**

The NSFT was performed as previously described (Greene et al. 2009). After the SPT, rats were deprived of food for 12 h, and on day 23 were placed in an open field (76.5 cm × 76.5 cm × 40 cm, Plexiglas) with a small amount of food in the centre. Rats were allowed to explore the open field for 8 min, and were filmed by a camcorder from above. The latency to feed (the time it took for rats to approach and take the first bite of the food) was recorded offline. Home-cage food intake was also measured as a control.

**Forced Swim Test (FST)**

On day 24, rats were subjected to a 10-min FST, a test where CUS has been shown to increase immobility (Greene et al. 2009). In this 1-d FST protocol, a rat was placed in a Plexiglas cylinder (65 cm height, 30 cm diameter) filled with 25 °C water to a height of 45 cm. After the test, animals were dried under a lamp for 30 min. The water was changed and the cylinder was rinsed with clean water after each swim. All FST experiments were filmed by a camcorder and only the first 5 min of the second swim was scored offline to obtain time spent immobile by an experienced rater blind to the experimental design (Greene et al. 2009; Warner-Schmidt & Duman, 2007). Immobility was defined as floating or remaining motionless without leaning against the wall of the cylinder.

**BDNF protein detection**

For the lamotrigine experiment without surgery, rats were killed by decapitation on day 24, and both halves of the frontal cortex and the hippocampal tissues were dissected out for protein assays. After homogenization by sonication, BDNF protein level of each sample was quantified by enzyme-linked immunosorbent assay (ELISA; R&D Systems, USA) according to the manufacturer’s instructions. BDNF protein level was normalized to the total protein level determined by BCA analysis (Pierce, USA). Results were expressed as picogram showing the mass of BDNF protein per mg total protein.

**Statistical analysis**

All analyses were performed using SPSS 13.0 software (SPSS Inc., USA) and data were reported as mean ± S.E.M. Analysis of variance (ANOVA) followed by Fisher’s *post-hoc* test was conducted when the interaction between two variables (stress and drug, infusion...
The effect of stress was significant \( F(1, 20) = 9.29, p < 0.01 \). The level of statistical significance was set at \( p < 0.05 \).

**Results**

**Chronic lamotrigine treatment ameliorates CUS-induced behavioural deficits**

Chronic treatment of lamotrigine produced a robust antidepressant effect in both SPT and NSFT (Fig. 2). For SPT (Fig. 2a), the interaction between stress condition (CUS or non-stressed) and drug treatment (lamotrigine or vehicle) was significant \( F(1, 20) = 12.39, p < 0.01 \). Further analyses indicated that CUS-treated rats receiving lamotrigine show a significant increase in sucrose preference compared to the CUS group receiving vehicle control \( (p < 0.01) \), to a level comparable to that of non-stressed control rats \( (p = 0.93) \). In addition, lamotrigine did not affect sucrose preference in non-stressed (NS) control rats \( (p = 0.74) \). There was no significant difference in total fluid consumption during the 1-h test. Total fluid consumption (ml) was expressed as mean \( \pm \) S.E.M. (NS + vehicle: \( 5.6 \pm 0.7 \); NS + lamotrigine: \( 6.3 \pm 1.2 \); CUS + vehicle: \( 4.9 \pm 1.3 \); CUS + lamotrigine: \( 5.1 \pm 0.9 \)).

Similar results were observed in the NSFT (Fig. 2b). The interaction between stress condition and drug treatment was significant \( F(1, 20) = 6.97, p < 0.05 \). The effect of stress was significant \( F(1, 20) = 6.82, p < 0.05 \), indicating CUS produced anxiogenic effects in rats. The effect of drug was also significant \( F(1, 20) = 10.03, p < 0.01 \). Further analyses indicated that CUS-treated rats receiving lamotrigine show a significant decrease in latency to feed compared to the CUS group receiving the vehicle control \( (p < 0.01) \), to a level comparable to that in non-stressed control rats \( (p = 0.72) \). In addition, lamotrigine did not affect latency to feed in non-stressed control rats \( (p = 0.98) \). There was no significant difference in home-cage food consumption and data are expressed as mean \( \pm \) S.E.M. (g) (NS + vehicle: \( 3.8 \pm 0.6 \); NS + lamotrigine: \( 4.2 \pm 0.4 \); CUS + vehicle: \( 4.1 \pm 0.7 \); CUS + lamotrigine: \( 3.5 \pm 0.8 \)).

**Chronic lamotrigine treatment restores CUS-induced decrease of BDNF expression**

Chronic treatment of lamotrigine significantly elevated frontal and hippocampal BDNF protein levels in both naive and CUS-treated animals (Fig. 3). The frontal and hippocampal BDNF levels exhibited a similar pattern. The results showed that the interaction between drug treatment and stress condition was significant in both frontal cortex (FC) \( F(1, 20) = 4.91, p < 0.05 \) and hippocampus (HC) \( F(1, 20) = 8.99, p < 0.01 \). The effect of stress was significant in both FC \( F(1, 20) = 26.12, p < 0.001 \) and HC \( F(1, 20) = 35.7, p < 0.001 \), indicating CUS significantly reduced BDNF expression in these two brain regions. The effect of drug was also significant in both FC \( F(1, 20) = 42.41, p < 0.001 \) and HC \( F(1, 20) = 55.61, p < 0.001 \). Further analyses indicated that CUS-treated rats receiving lamotrigine show a significant increase of BDNF protein levels compared to the CUS group receiving saline control (for both FC and HC, \( p < 0.001 \)), to a level comparable to that of non-stressed control rats (FC, \( p = 0.27 \); HC, \( p = 0.29 \)). Lamotrigine also increased BDNF levels in non-stressed rats (FC, \( p < 0.05 \); HC, \( p < 0.01 \)).
**The blockade of BDNF-TrkB signalling abolishes the antidepressant effects of lamotrigine**

The blockade of BDNF-TrkB signalling by the TrkB inhibitor K252a completely abolished the antidepressant effects of lamotrigine in SPT, NSFT and FST of CUS-treated rats (Fig. 4). For SPT (Fig. 4a), the interaction between infusion (K252a or DMSO) and injection (lamotrigine or vehicle) was significant \[F(1,20) = 15.87, p < 0.01\]. The effect of infusion was significant \[F(1,20) = 7.35, p < 0.05\], as was the effect of injection \[F(1,20) = 19.49, p < 0.001\]. Further analyses indicated that consistent with the experiment without surgery, CUS-treated rats receiving lamotrigine show a significant increase in sucrose preference compared to the CUS group receiving vehicle control \([p < 0.001]\). The antidepressant effect of lamotrigine was completely blocked by infusion of the BDNF TrkB receptor antagonist K252a; the CUS + lamotrigine + K252a group was not significantly different from the CUS + vehicle + DMSO group \((p = 0.74)\) or the CUS + vehicle + K252a group \((p = 0.82)\). There was no significant difference in home-cage food consumption (data not shown).

For the NSFT (Fig. 4b), the interaction between infusion and injection treatment was significant \[F(1,20) = 11.36, p < 0.01\]. Further analyses indicated that similarly to the experiment without surgery, CUS-treated rats receiving lamotrigine show a significant decrease in latency to feed compared to the CUS group receiving vehicle control \([p < 0.01]\).

For the FST (Fig. 4c), the interaction between infusion and injection treatment was significant \[F(1,20) = 10.36, p < 0.01\]. Further analyses indicated that CUS-treated rats receiving lamotrigine show a significant decrease in immobility compared to the CUS group receiving vehicle control \([p < 0.001]\). This antidepressant effect of lamotrigine was completely blocked by infusion of the TrkB antagonist K252a; the CUS + lamotrigine + K252a group was not significantly different from the CUS + vehicle + DMSO group \((p = 0.74)\) or the CUS + vehicle + K252a group \((p = 0.84)\).

**Discussion**

The present study provides further evidence that the mood stabilizer lamotrigine produces antidepressant effects in rodent models of depression. Chronic daily administration of lamotrigine produces antidepressant effects in CUS-treated animals in SPT and NSFT. Chronic lamotrigine treatment also up-regulates frontal and hippocampal BDNF levels in both non-stressed and CUS-treated animals, and more importantly, restores stress-induced down-regulation of BDNF expression.
BDNF to the same level as non-stressed vehicle-treated control animals. Finally, pharmacological inhibition of BDNF-TrkB signalling completely abolishes the antidepressant effects of lamotrigine in SPT, NSFT and FST.

As previously mentioned, it is the dose of lamotrigine that determines whether it exerts antidepressant function or not in rodent models of depression. Low doses (1–5 mg/kg) of lamotrigine fail to reduce immobility in mice FST (Ali et al. 2003). In contrast, when given at higher doses (10–32 mg/kg), acute and subchronic (3 d) lamotrigine treatments produce significant antidepressant actions in the FST (Bourin et al. 2005; Li et al. 2010; Prica et al. 2008). In our previous study, subchronic (7 d) 30 mg/kg daily treatment of lamotrigine reduced escape failures in the LH model of depression (Li et al. 2010). This dose is well tolerated and does not change locomotor activities significantly (Li et al. 2010). Thus, we continued to use this dose in the present study.

Previous studies on lamotrigine mainly used FST and LH paradigms, which respond to acute or subchronic antidepressant treatments, and thus have low face validities. In the present study, we utilized two rodent models that are responsive to chronic antidepressant treatments, and better mimic the therapeutic time-course in clinical studies. CUS is generally considered as a good preclinical model of depression (Schmidt & Duman, 2007). After CUS, animals develop a spectrum of behavioural changes that resemble symptoms of patients with major depressive disorder (Willner, 2005). One of the most salient abnormalities is reduced motivation for incentivizing stimuli, such as sucrose solutions, resembling the symptom of anhedonia in depressive patients. NSFT is a measure of anxiety-like behaviour, and is also responsive to chronic antidepressant treatments (Schmidt & Duman, 2007). In our study, CUS-treated rats showed severe anhedonic (decreased sucrose preference) and anxiogenic (increased latency to feed in a novel environment) symptoms, both of which were significantly ameliorated by chronic lamotrigine treatment. In the non-stressed control group, lamotrigine did not cause any significant behavioural changes. In the NSFT, lamotrigine did not affect home-cage food intake, indicating the effects observed were not the results of some non-specific changes such as hunger level. Taken together, lamotrigine has demonstrated antidepressant actions in various rodent models of depression.

Consistent with previous studies (Chang et al. 2009; Li et al. 2010), we found that chronic treatment of lamotrigine significantly increased frontal and hippocampal BDNF protein levels in rats. A potential target of lamotrigine in the brain is the arachidonic acid signalling cascade. Chronic administration of lamotrigine markedly down-regulates arachidonic acid signalling (Rao & Rapoport, 2009). This signalling pathway has been implicated in interfering with transcription of neuronal survival factors, and its down-regulation enhances the expression of BDNF and other neurotrophic factors in the brain (Garrido et al. 2003).

Importantly, chronic lamotrigine administration restores CUS-down-regulated BDNF expression to comparable levels as in non-stressed vehicle-treated control groups. The neurotrophic hypothesis of depression suggests that stress and depression reduce levels of neurotrophic factors in several key brain regions including the frontal cortex and hippocampus,
leading to morphological and functional abnormalities and this deficiency can be reversed by antidepressant treatments (Duman, 2004). In animals subjected to CUS, the frontal and hippocampal BDNF levels are significantly reduced and can be alleviated by chronic treatments of antidepressants (Duman & Monteggia, 2006; Nibuya et al. 1999). Given that both bipolar disorder and major depressive disorder patients have reduced hippocampal levels of BDNF and other neurotrophic factors (Dwivedi et al. 2003; Knable et al. 2004), up-regulation of BDNF might be a common mechanism for antidepressants to exert therapeutic effects.

To directly address this hypothesis, we pharmacologically blocked BDNF-TrkB signalling in the brain by infusing the TrkB receptor antagonist K252a. In the adult brain, BDNF preferentially binds to the tyrosine kinase receptor TrkB (Duman & Monteggia, 2006). The blockade of BDNF-TrkB signalling in hippocampus by the tyrosine kinase inhibitor K252a abolished the antidepressant-like effects of BDNF in the LH model of depression (Shirayama et al. 2002). In the present study, we found that K252a blocks the behavioural effects of lamotrigine in SPT, NSFT and FST, indicating that activation of BDNF-TrkB signalling is required for antidepressant actions of lamotrigine. Furthermore, when administered during the last week of the chronic treatment of lamotrigine, K252a was able to completely reverse its antidepressant actions. Consistent with the neurotrophic hypothesis of depression (Duman, 2004), our current findings indicate that the maintenance of sustained neurotrophic mechanisms such as the activation of the BDNF signalling pathway is required for the behavioural effects of lamotrigine.

There are a few limitations in the current experiment which should be addressed in future studies. The number of animals used could be increased to obtain more satisfactory statistical power. Furthermore, the specificity of the TrkB inhibitor K252a is not optimal, as it also inhibits other tyrosine kinase receptors from the same family such as TrkA, as well as serine/threonine kinases (Shirayama et al. 2002). Inhibitors with higher selectivity, such as the BDNF scavenger TrkB-Fc, can help provide more information. Moreover, transgenic mouse models such as conditional forebrain BDNF knockout mice can also be valuable tools in elucidating the role of the BDNF pathway in mediating the behavioural effects of lamotrigine. In addition, the i.c.v. route of inhibitor infusions makes it difficult to determine the regional specificity of BDNF signalling required for lamotrigine. Based on the present study and previous studies, we suggest that the frontal cortex and the hippocampus could be two potential brain regions involved, and future studies with local microinjection are needed to address this issue.

Taken together, the present study shows that chronic treatment of lamotrigine produces antidepressant effects in CUS/SPT, NSFT and ameliorates CUS-induced down-regulation of BDNF in frontal cortex and hippocampus. The blockade of BDNF-TrkB signalling abolishes behavioural actions of lamotrigine in SPT, NSFT and FST. The present study strongly suggests that lamotrigine exerts its antidepressant actions by regulating the BDNF-TrkB signalling cascade.

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Statement of Interest

None.

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