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An inhibitor of casein kinase 1 ε/δ partially normalizes the manic-like behaviors of the *Clock*Δ19 mouse

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Abstract

Bipolar disorder is a terrible and debilitating disease with limited treatment options. Circadian rhythm disruptions are prominent in bipolar subjects, and studies have shown that rhythm stabilization through psychosocial interventions can improve their symptoms. Furthermore, mice with a mutation in one of the central circadian proteins, CLOCK, have severely disrupted rhythms along with a behavioral profile that closely resembles human mania. A compound has been developed (CK01, similar to PF-670462) that inhibits the activity of casein kinase 1 (CK1), a critical protein involved in the timing of the molecular clock. Previous studies have shown that PF-670462 and other similar compounds are capable of entraining and stabilizing rhythms in arrhythmic animals. Here we show that chronic administration of CK01 leads to a reversal of the anxiety-related behavior, and partial reversal of the depression-related phenotypes of the *Clock* mutant mouse. This drug had no significant effects on the behavior of wild-type mice at the doses tested. These results suggest that CK1ε/δ inhibitors could be viable drugs for the treatment of bipolar disorder.

Keywords

anxiety; bipolar disorder; circadian rhythms; depression; mouse

Introduction

Bipolar disorder is a devastating disease that is characterized by periods of depression and mania. Although medications like lithium are effective in a number of individuals, the current mood stabilizers cause a number of significant side-effects and do not alleviate symptoms in individuals with this disorder. Thus, there is a need to develop better treatments. Several recent studies point towards disruptions in the circadian system as a strong contributing factor to the development of bipolar disorder (McClung, 2007). Psychosocial therapies such as interpersonal and social rhythm therapy have demonstrated that regulation of the circadian cycle leads to improved patient outcomes in a shorter time than traditional psychotherapy (Frank *et al.*, 2000). Recently, several drugs have been designed to inhibit casein kinase 1 ε/δ (CK1ε/δ), critical proteins involved in the regulation of molecular rhythms. Circadian rhythms are controlled by a transcriptional/translational

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Conflicts of interest
There are no conflicts of interest.

feedback loop that cycles over the course of 24 h (Ko and Takahashi, 2006). The CLOCK and BMAL1 proteins are the central transcriptional activators that regulate the expression of the *Period* (*Per*) and *Cryptochrome* (*Cry*) genes. These proteins then feed back and inhibit the activity of CLOCK/BMAL1. CK1ε/δ phosphorylates the PER proteins, leading to the recruitment of F-box protein β-TRCP, which then targets the PER proteins for degradation (Eide *et al.*, 2005; Shirogane *et al.*, 2005; Shanware *et al.*, 2011). This action helps regulate the timing of the molecular rhythms. A specific CK1ε/δ inhibitor, PF-670462, which inhibits CK1ε/δ with a potency in the nanomolar range, has been developed previously (Badura *et al.*, 2007). Studies have shown that PF-670462 inhibits PER3 nuclear translocation, and also leads to a dose-dependent phase delay in rhythms when administered chronically to wild-type (WT) rats in a light/dark cycle (Badura *et al.*, 2007; Sprouse *et al.*, 2010). These results were reproduced in diurnal cynomolgus monkeys, suggesting that they would have the same effect on humans (Sprouse *et al.*, 2009). Recently, Meng *et al.* (2010) reported that the administration of PF-670462 could entrain mice with disrupted rhythms caused either by constant light or a mutation in the *Vipr2* gene (Meng *et al.*, 2010). These results suggest that PF-670462 or other CK1ε/δ inhibitors could be used as therapeutic tools to entrain the rhythms of individuals with rhythm disruptions.

Our lab previously reported that mice with a mutation in the *Clock* gene (*Clock*Δ19 mice, described in King *et al.*, 1997) have a behavioral profile that is strikingly similar to human mania (King *et al.*, 1997; Roybal *et al.*, 2007). This includes hyperactivity, decreased anxiety (i.e. increased exploratory drive), decreased depression-like behavior, and a hyperhedonic response to rewarding stimuli (McClung *et al.*, 2005; Roybal *et al.*, 2007). Lithium treatment is able to restore the majority of these abnormal behavioral phenotypes to WT levels (Roybal *et al.*, 2007), suggesting that this mouse can be used to screen additional compounds that might be effective mood-stabilizing drugs. We aimed to determine whether a CK1ε/δ inhibitor (CK01), a compound with a highly similar structure and function to PF-670462, would be able to restore normal behavior to the *Clock*Δ19 mouse. A positive result suggests that CK1ε/δ inhibitors may be effective compounds for rhythm and mood stabilization in bipolar subjects.

Methods

Subjects and housing

*Clock*Δ19 mutant mice were created by *N*-ethyl-*N*-nitrosourea mutagenesis and produce a dominant-negative CLOCK protein defective in transcriptional activation activity as described (King *et al.*, 1997). For all experiments using *Clock*Δ19 mutants, 8–10-weeks-old adult male mutant (*Clock/Clock*; Mut) and WT (+/+) littermate controls on a mixed BALBc; C57BL/6 background were used. Mice were group housed in sets of two to four per cage on a 12:12 h light/dark cycle (lights on at 06:00 h, lights off at 18:00 h) with food and water freely available.

Lithium administration

Lithium-treated mice received 600 mg/l of LiCl in drinking water for 10 days before behavioral testing, and throughout the course of the testing. This administration results in a stable serum concentration of lithium in the low therapeutic range for humans (0.41±0.06 mmol/l), with little to no adverse health consequence (Roybal *et al.*, 2007).

CK01 preparation and administration

CK01 was dissolved in 20% sulfobutyl ether β-cyclodextrin in sterile water at three doses (5.6, 17.8, 32.0 mg/kg). Approximately 4.8 microliters of 1N HCl was added per milligram of CK01 used to the vehicle solution. The CK01 solution was injected daily subcutaneously

for 10 days before behavioral testing, and daily injections continued throughout testing. The compound was administered at 06:30–07:00 h (Zeitgeber time 1) each day. The 5.6 mg/kg dose led to no changes in behavior throughout the study (data not shown) and thus results are presented for the higher doses only.

Behavioral assays

Locomotor response to novelty—Mice were placed into individual automated locomotor activity chambers that were equipped with infrared photobeams (San Diego Instruments, San Diego, California, USA). Activity measurements commenced upon the first beam break and were measured continuously, with data collected in 5-min blocks over a period of 2 h.

Elevated plus maze—The plus maze apparatus consisted of closed and open arms (all arms are 30×5 cm, with 25cm tall walls on the closed arms). Mice were placed in the center of an elevated plus maze and the time spent in the open arms, closed arms, and center of the maze, along with the number of entries into the open and closed arms of the maze were determined using Ethovision 3.0 video tracking software (Noldus, Leesburg, Virginia, USA). The time spent on the open arms and the percent of entries into the open arms were used to determine the anxiety-related behavior. The plus maze apparatus was sanitized and allowed to dry before each mouse was tested.

Dark/light test—The dark/light apparatus is a two-chambered box (25×26 cm for each side; Med Associates, St Albans, Vermont, USA), one side of which was kept dark and the other side was brightly lit by a fluorescent bulb at the top of the chamber. Mice were allowed to habituate to the dark side of the box for 2 min. Following the habituation period, the door between the compartments was opened and they were allowed to explore freely on both sides of the apparatus for 10 min. Anxiety-like behavior was measured as the percent of time spent in the light side.

Forced swim test—Mice were placed in 4-liter Pyrex glass beakers filled with 3 liters of water at 21–25°C for 6min. All test sessions were recorded from the side of the beakers by a video camera. Water was changed between subjects. The video was analyzed and scored by an observer blind to the genotypes and treatment groups. After a 2-min habituation period, latency to immobility was determined as the first cessation of movement. Total immobility was measured during the last 4 min of the test and was measured as the time spent without movement except for a single limb paddling to maintain flotation.

Statistics

All data were analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc tests, using GraphPad Prism 5 statistical software for Windows (GraphPad Software Inc., La Jolla, California, USA). All data are presented as means±SEM with P value less than 0.05 considered statistically significant.

Results

CK01 has no effect on general locomotor activity

To determine the effects of CK01 administration on manic-like behaviors, *Clock*Δ19 mice and WT littermates received 10 days of systemic CK01 at three doses (5.6, 17.8, and 32.0 mg/kg) or sulfobutyl ether-β-cyclodextrin, and were compared with *Clock*Δ19 and WT mice receiving lithium treatment, following a battery of behavioral tests. As the 5.6mg/kg dose led to no discernable behavioral effects on any measure, results are shown only for the

two higher doses. Mice were first assessed for their locomotor response to novelty. As reported previously (Roybal *et al.*, 2007), *Clock*Δ19 mice are hyperactive when compared with WT animals (Fig. 1), and CK01 administration had no significant effect on locomotor activity, similar to mice receiving lithium treatment (Fig. 1). Because treatment had no effect on locomotor activity, effects on the other behavioral tests are interpreted as changes in anxiety-related and depression-related behavior.

CK01 normalizes the anxiolytic behavior of the *ClockΔ19* mice

To examine the effects of CK01 administration on anxiety-related behavior, mice were subjected to two different measures: the elevated plus maze and the dark/light test. In the elevated plus maze, the excess exploratory behavior of *Clock*Δ19 mice was rescued by the 32.0 mg/kg dose of CK01, as seen by a reduction in the amount of time spent in the open arms of the maze, similar to lithium treatment (Fig. 2a). The 17.8 mg/kg dose also caused a near-significant decrease in open-arm time (Fig. 2a). In the dark/light test, CK01 also had anxiogenic effects similar to lithium treatment (Fig. 2b). A significant decrease in time spent in the light side of the light/dark box was observed following administration of 17.8 mg/kg CK01 and a more robust decrease was observed following 32.0 mg/kg CK01 treatment. CK01 treatment had no detectable effect on WTanimals in any measure of anxiety-related behavior (Fig. 2a and b); however, lithium treatment had a slight anxiolytic effect on open-arm time in the elevated plus maze when compared with the 32.0 mg/kg dose of CK01, but did not differ significantly from either vehicle or the 17.8 mg/kg dose of CK01.

CK01 treatment partially normalizes the antidepressant effects of the *ClockΔ19* mouse

In the forced swim test, *Clock*Δ19 mice displayed a significant decrease in depression-related behavior, as described previously (Fig. 3a; Roybal *et al.*, 2007). Unlike lithium treatment, which normalizes the effects on depression-related behavior by causing an increase in total immobility time (Fig. 3a), CK01 treatment had no significant effect on immobility time. However, CK01 treatment did cause a significant decrease in latency to the first bout of immobility in *Clock*Δ19 mice at both doses, without affecting WT animals, suggesting a partial reversal of this phenotype (Fig. 3b).

Discussion

Our results show that CK01 treatment leads to a reversal of the abnormal anxiolytic behaviors of the *Clock*Δ19 mouse, which were more robust following the administration of a higher dose (32.0 mg/kg). Furthermore, there was a partial reversal of the antidepressant phenotype. Along with other abnormal circadian and reward-related phenotypes, these behaviors constitute a profile of abnormal behavioral responses in the *Clock*Δ19 mouse which together represent a manic-like phenotype reminiscent of human bipolar disorder. Interestingly, CK01 treatment does not reverse the hyperactivity in a novel environment that is prominent in the *Clock*Δ19 mouse. Lithium treatment also does not reverse this phenotype, and recent studies in our lab suggest that treatment with another mood-stabilizing agent, valproate, also has no effect on this particular behavior (unpublished observations). These results suggest that the hyperactivity in the *Clock*Δ19 mouse is controlled by a separate mechanism that is independent of the control of anxiety-related and mood-related behavior. This separation of mechanisms is particularly relevant as amphetamine-induced and other psychostimulant-induced locomotor activity is often used as a model of mania. Indeed, separate drugs may be needed to reverse specific endophenotypes of bipolar illness. Interestingly, a recent report found that PF-670462 does normalize amphetamine-induced hyperactivity likely through a regulation of Darpp-32-PP1-GlurR1 signaling in the nucleus accumbens (NAc) (Li *et al.*, 2011). This suggests that CK1

inhibitors may be able to modulate certain behavioral abnormalities through circadian clock stabilization and others through effects on modulation of NAc output.

Previous studies have found that CK01 treatment leads to phase delays and a lengthening of the period of WTanimals while it entrains the rhythms of animals that are arrhythmic (Meng *et al.*, 2010). CK1δ inhibition leads to a daily enhancement of PER protein in the nucleus of the cell, which presumably results from decreased degradation of the PER proteins or enhanced nuclear translocation. In the *Clock*Δ19 mice, the PER protein levels are very low and rhythms in a light/dark cycle are sometimes weak (Vitaterna *et al.*, 2006). Future studies will determine whether CK01 stabilizes the rhythms in these mice through increased PER protein concentrations in the suprachiasmatic nucleus. This rhythm stabilization could have therapeutic effects in the *Clock*Δ19 mice by affecting rhythms in the ventral tegmental area dopamine neurotransmission. Indeed, virtually all aspects of dopaminergic transmission have a circadian rhythm, and these rhythms are disrupted in the *Clock*Δ19 mice (McClung *et al.*, 2005). It is possible that the administration of CK01 may normalize the rhythm of dopaminergic transmission and thus normalize the *Clock*Δ19 behavior. Future studies will determine whether CK01 does indeed alter rhythms in the ventral tegmental area dopaminergic transmission. CK01 may also normalize *Clock*Δ19 anxiety-related behavior by stabilizing rhythms in the amygdala, which is known to be an important regulator of these behaviors (Davis, 1992).

It is unclear why there was only a partial reversal of depression-related behavior in the *Clock*Δ19 mice in the forced swim test, in that the latency to immobility was significantly altered but the total time immobile was not. It is possible that a higher dose or more chronic treatment paradigm would be sufficient to alter both parameters of this measure. It is also possible that CK1 proteins have a more prominent role in the control of anxiety. Nevertheless, the results are promising and suggest that CK1ε/δ inhibitors might be able to normalize both anxiety-related and mood-related phenotypes in humans.

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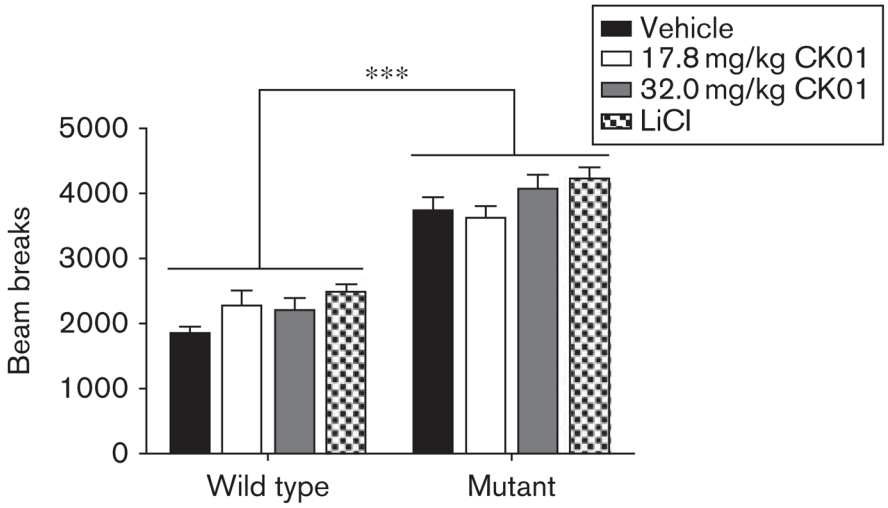

Fig. 1.

CK01 administration has no effect on the locomotor response to novelty. Locomotor activity was measured in the *Clock*Δ19 mice and the wild-type littermates for 2 h following 10 days of CK01 or lithium administration. Analysis by two-way analysis of variance revealed a significant main effect of genotype [$F_{(1,90)}$ =145.94, P<0.001]. Bonferroni's post-hoc tests revealed that there was no significant effect of any treatment on locomotor response to novelty (n=12–14/group). LiCl, lithium chloride. ***P<0.001.

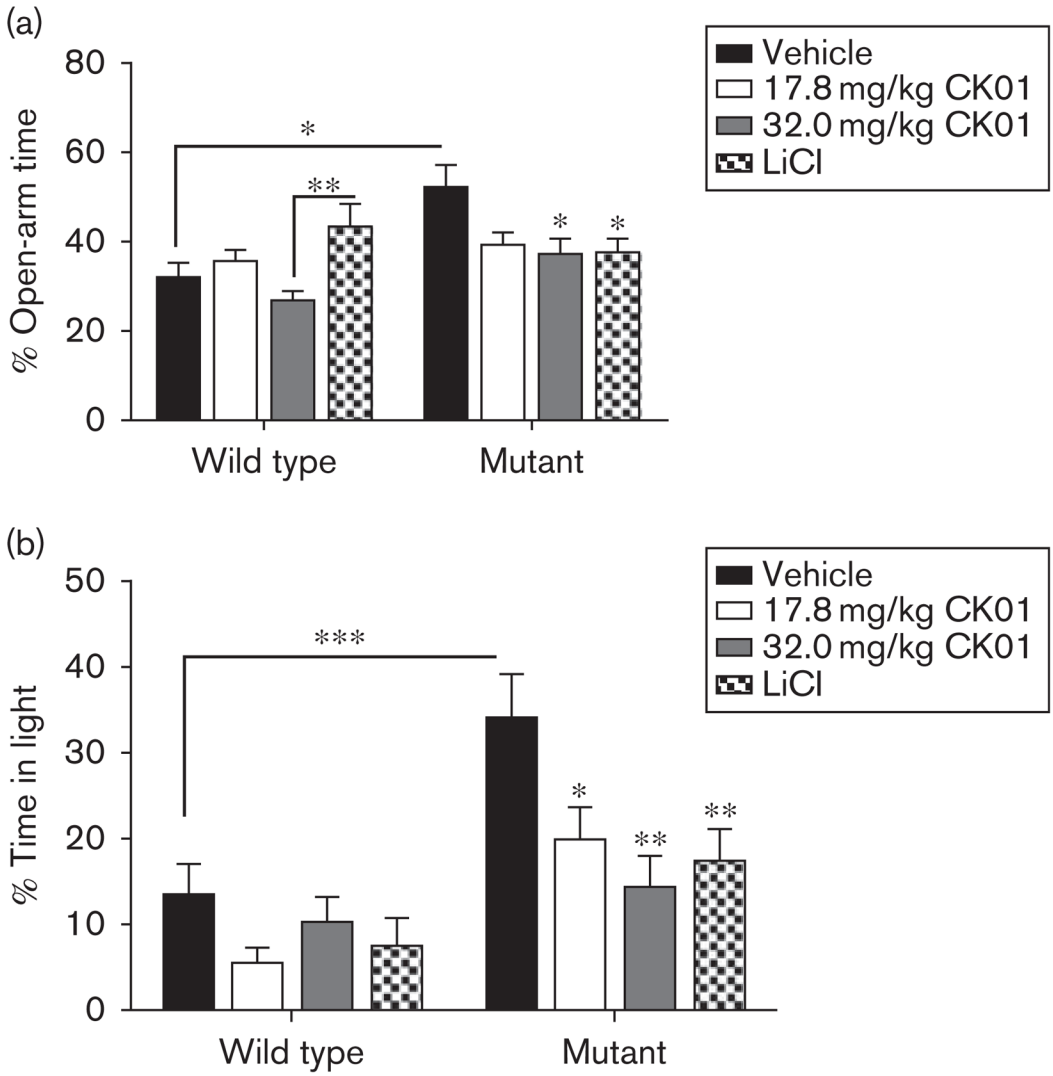

Fig. 2.

CK01 administration normalized anxiety-related behavior in the *Clock*Δ19 mice. (a, b) The *Clock*Δ19 mice and the wild-type (WT) littermates were assessed for anxiety-related behavior following CK01 and lithium administration in both the elevated plus maze (EPM) (a) and dark/light test (b). Analysis by two-way analysis of variance revealed that *Clock*Δ19 mice display previously reported decreased anxiety-related behavior by spending more time in the open arms of the EPM [main effect of genotype; $F_{(1,83)}$ =6.39, P<0.02] and light side of the dark/light box [main effect of genotype; $F_{(1,85)}$=21.93, P<0.001]. Bonferroni's post-tests revealed that 17.8 mg/kg CK01 treatment caused a reduction in open arm time that did not reach significance in the EPM (a) and a significant reduction in time spent in the light side of the dark/light test (b, t =2.553, P<0.05). 32.0 mg/kg CK01 treatment in *Clock*Δ19 mice caused a significant decrease in EPM open arm time (a, t=2.55, P<0.05) and time spent on the light side of the dark/light test (b, t=3.62, P<0.01). As described previously, lithium had antimanic effects on the *Clock*Δ19 mice by restoring open-arm time in the EPM (a, t=2.75, P<0.05) and time in the light side of the dark/light test (b, t=3.25, P<0.05) to WT levels. CK01 treatment had no significant effect on the anxiety-related behavior when compared with the vehicle-treated WT mice; however, Bonferroni's post-hoc tests revealed

that lithium treatment caused an anxiolytic effect that differed significantly from mice receiving 32.0 mg/kg CK01 (a, $t=3.04$, $P<0.01$), but not vehicle or 17.8 mg/kg CK01 treated mice. LiCl, lithium chloride. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

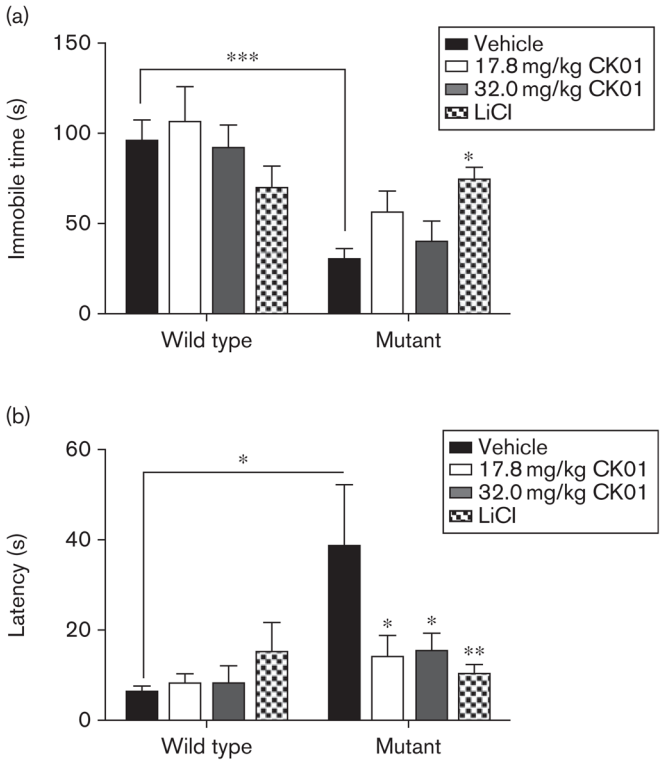

Fig. 3.

CK01 administration has partial effects on *Clock*Δ19 depression-related behavior. (a) *Clock*Δ19 and wild-type (WT) mice were assessed for depression-related behavior using the forced swim test following CK01 and lithium treatment. Analysis by two-way analysis of variance (ANOVA) showed that the *Clock*Δ19 mice have decreased immobility time compared with the WT animals [main effect of genotype; $F_{(1,88)}$ =21.39, P<0.001]. Bonferroni's post-hoc tests showed that lithium treatment had previously reported antimanic effects on *Clock*Δ19 depression-related behavior by increasing the total immobility time (t=2.52, P<0.05). CK01 treatment had no detectable effect on either *Clock*Δ19 or WT immobile time. (b) Latency to immobility was assessed following CK01 and lithium treatment in *Clock*Δ19 and WT animals. *Clock*Δ19 mice had a significantly longer latency to immobility than WT animals by two-way ANOVA [main effect of genotype; $F_{(1,87)}$ =6.30, P<0.02]. Bonferroni's post-hoc tests revealed a significant effect of treatment with 17.8 mg/kg CK01 (t=2.82, P<0.05), 32.0 mg/kg CK01 (t =2.80, P<0.05), and lithium (t=3.40, P<0.01) treatment on latency to immobility in *Clock*Δ19 mice. WT animals were not significantly affected by treatment. LiCl, lithium chloride. *P<0.05, **P<0.01, ***P<0.001.