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ATTENUATION OF MANIA-LIKE BEHAVIOR IN NA$^+$,K$^+$-ATPASE α3 MUTANT MICE BY PROSPECTIVE THERAPIES FOR BIPOLAR DISORDER: MELATONIN AND EXERCISE

G. S. KIRSHENBAUM,[a,b]* C. R. BURGESS,[c] N. DÉRY,[d] M. FAHNESTOCK,[e] J. H. PEEVER[c] AND J. C. RODER[a,b,f]

[a] *Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada*

[b] *Institute of Medical Science, University of Toronto, Toronto, Ontario M5S 1A8, Canada*

[c] *Cell and System Biology, University of Toronto, Toronto, Ontario M5S 3G3, Canada*

[d] *Department of Psychology, Neuroscience and Behavior, McMaster University, Hamilton, Ontario L8N 3Z5, Canada*

[e] *Department of Psychiatry and Behavioral Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5, Canada*

[f] *Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada*

Abstract—Bipolar disorder is a neuropsychiatric disease characterized by states of mania with or without depression. Pharmacological treatments can be inadequate at regulating mood for many individuals. Melatonin therapy and aerobic exercise are independent prospective therapies for bipolar disorder that have shown potential as mood stabilizers in humans. *Myshkin* mice (*Myk*/+) carry a heterozygous missense mutation in the neuronal Na$^+$,K$^+$-ATPase α3 and model mania-related symptoms of bipolar disorder including increased activity, risk-taking behavior and reductions in sleep. One cohort of *Myk*/+ and wild-type littermates (+/+) was treated with melatonin and a separate cohort was treated with voluntary exercise. Mania-related behavior was assessed in both cohorts. The effect of melatonin on sleep and the effect of exercise on brain-derived neurotrophic factor (BDNF) expression in the hippocampus were assayed. Melatonin and voluntary wheel running were both effective at reducing mania-related behavior in *Myk*/+ but did not affect behavior in +/+. Melatonin increased sleep in *Myk*/+ and did not change sleep in +/+. *Myk*/+ showed higher baseline levels of BDNF protein in the hippocampus than +/+. Exercise increased BDNF protein in +/+ hippocampus, while it did not significantly affect BDNF levels in *Myk*/+ hippocampus. These findings support initial studies in humans indicating that melatonin and exercise are useful independent adjunct therapies for bipolar disorder. Their effects on mood regulation should be further examined in randomized clinical trials. Our results also suggest that hippocampal BDNF may not mediate the effects of exercise on mania-related behavior in the *Myk*/+ model of mania.

Key words: Na$^+$,K$^+$-ATPase α3, Atp1a3, mania, bipolar disorder, melatonin, voluntary exercise.

INTRODUCTION

Bipolar disorder is a complex neuropsychiatric disease defined by the presence of one or more states of mania with or without depression (American Psychiatric Association, 1994). The mood stabilizers lithium and valproic acid are the primary treatment for bipolar disorder, as well as atypical antipsychotics and antidepressants (Tohen et al., 1999; Sachs et al., 2007). In many cases mood stabilizers can be ineffective at treating the most debilitating symptoms of disease and facilitating long-term maintenance of a balanced mood (Judd and Akiskal, 2003; Kupfer, 2005). 20–30% of affected individuals do not respond to treatment (Souery et al., 2006), while 25–50% of individuals that respond to mood stabilizers do not experience adequate periods of remission (Soares and Young, 2007). Nonetheless, lithium and valproic acid remain the best pharmacological or non-pharmacological treatment option for bipolar disorder.

Initial studies in humans indicate that melatonin and agomelatine, a melatonin agonist, are effective add-on treatments to mood stabilizers (Bersani and Garavini, 2000; Gao and Calabrese, 2005; Calabrese et al., 2007). Accordingly, melatonin receptors are being investigated as novel targets for bipolar disorder (Turek and Gillette, 2004). Separate studies in humans suggest that exercise, in combination with mood stabilizers, improve outcomes in bipolar disorder (Ng et al., 2007; Kucyi et al., 2010; Sylvia et al., 2011), and routine exercise is a common practice in high-functioning bipolar individuals (Murray et al., 2011). Exercise increases hippocampal levels of brain-derived neurotrophic factor (BDNF) (Vaynman et al., 2004), which may mediate improvements in mood (Hashimoto, 2010; Zoladz and Pilc, 2010; Fernandes et al., 2011). Melatonin agonists and exercise are distinct promising

*Correspondence to: G. S. Kirshenbaum, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Room 860, Toronto, Ontario M5G 1X5, Canada. Tel: +1-917-843-7995; fax: +1-212-543-5474.

E-mail addresses: greershelby@gmail.com, gsk2125@columbia.edu (G. S. Kirshenbaum).

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; EDTA, ethylenediaminetetraacetic acid; EEG, electroencephalographic; ELISA, enzyme-linked immunosorbent assay; EMG, electromyographic; ENU, N-ethyl-N-nitrosourea; EPM, elevated plus maze; LDB, light–dark box; *Myk*, *Myshkin*; REM, rapid eye movement; RT-PCR, real-time polymerase chain reaction; SEM, standard error of mean.

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putative adjunct therapies for bipolar disorder that may lead to better control of mood.

We examined if heterozygous *Myshkin* (*Atp1a3*$^{Myk/+}$; *Myk*/+) mice, a putative model of mania (Kirshenbaum et al., 2011), respond to melatonin or voluntary exercise treatment. *Myk*/+ mice carry a missense mutation induced by *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis in the *Atp1a3* gene that encodes the neuron-specific Na^+,K^+-ATPase α3 isoform. The mutation results in a normally expressed but inactive enzyme leading to a 36–42% reduction in total Na^+,K^+-ATPase activity in the brain (Clapcote et al., 2009; Kirshenbaum et al., 2011). Melatonin and exercise may each independently regulate Na^+,K^+-ATPase activity by a direct or indirect mechanism (Oner et al., 2002; Ben et al., 2009; Lima et al., 2009a; Souza et al., 2009). Na^+,K^+-ATPase dysfunction is potentially involved in the pathophysiology of bipolar disorder. Bipolar individuals show reduced postmortem expression of Na^+,K^+-ATPase α2 in the temporal cortex and Na^+,K^+-ATPase α3 in the prefrontal cortex (Rose et al., 1998; Tochigi et al., 2008), and genetic associations between bipolar disorder and variants encoding Na^+,K^+-ATPase α1, α2 and α3 subunits are emerging (Mynett-Johnson et al., 1998; Goldstein et al., 2009). Moreover, bipolar individuals show abnormal regulation of endogenous ouabain-like compounds, which regulate Na^+, K^+-ATPase activity (Croyle et al., 1997; Grider et al., 1999; Goldstein et al., 2006; El-Mallakh et al., 2010).

Myk/+ mice exhibit mania-related behaviors that respond to lithium and valproic acid including; hyperactivity in the open field, risk-taking behavior in the elevated plus maze and light–dark box, and reductions in rapid eye movement (REM) and non-REM sleep (Kirshenbaum et al., 2011). We investigated if mania-related behavior in these mice could be attenuated by melatonin or voluntary exercise. If these treatments are effective in a mouse model of mania, they may prove useful in the management of mania in humans.

EXPERIMENTAL PROCEDURES

Mice

Myshkin mice were maintained by backcrossing to the C57BL/6NCr strain for 20 generations and were genotyped using a polymerase chain reaction assay, as previously described (Clapcote et al., 2009; Kirshenbaum et al., 2011). *Myshkin* mice were bred from female C57BL/6NCr mice and heterozygous *Myshkin* male mice. Littermates were used as controls for all experiments. All procedures were approved by the Animal Care Committee of the Toronto Centre for Phenogenomics and followed the Province of Ontario Animals for Research Act 1971 and requirements of the Canadian Council on Animal Care. Animals were housed in filtered cages containing nesting material at 21 ± 1 °C, under a 12:12-h light–dark cycle (lights on: 0700–1900 h) and 50–60% humidity. Pups of mixed genotypes were weaned at 4 weeks and housed by sex in groups of 3–5 animals. Sterile food (Harlan Teklad 2918) and water were provided *ad libitum*.

Melatonin treatment

Melatonin (Sigma Aldrich, St. Louis MO USA) solution was prepared in ethanol and dissolved in drinking water (12.5 μg/mL, in 0.066% ethanol). The solution was administered in a water bottle for 21 days prior to behavioral testing and continued throughout testing. Wild-type (+/+) and *Myk*/+ mice consume an average of 4.1 mL of water per day. Based on the melatonin concentration and water intake, the approximate dose of melatonin was 2 mg/kg/day which has been shown to be effective (Brooks and Peever, 2011). Water consumption in mice is primarily during the dark phase and this route of administration does not interfere with endogenous circadian rhythms (Brooks and Peever, 2011). Melatonin is light sensitive, so the water bottles were covered in foil and changed every 2 days. Control groups received identical water bottles with drinking water and 0.066% ethanol with regular bottle changes. One cohort of mice was used for behavioral testing while a separate cohort was used for sleep analysis.

Exercise treatment

Each cage contained a running wheel and dome (Bio Serve InnoDome and InnoWheel) for 42 days prior to behavioral testing and remained throughout testing. Mice in the wheel-treated group had *ad libitum* access to movable wheels, while the wheels were immobile for the control group. One cohort of mice was used for behavioral testing while a separate cohort was used for BDNF analysis.

Behavioral studies

Behavioral testing was performed during the light phase (0900–1500 h) on mice aged 6–12 weeks. Testing was conducted on balanced numbers of male and female mice; no sex differences were detected, so results were pooled. Prior to behavioral observation, animals were placed in a testing environment for 30 min for acclimatization. 70% ethanol solution and Clidox solution were used to clean surfaces and equipment between subjects. Behavior was scored by Observer 5.0 software (Noldus Information Technology, Wageningen, Netherlands) by an observer blinded to genotype and treatment. For all groups, the open field, elevated plus maze, and light–dark box tests were separated by 1 week. The order of the elevated plus maze and light–dark box was counterbalanced across groups, and order had no effect on behavior.

Open field

Mice were placed in the center of a transparent Plexiglas open field (41.25 cm × 41.25 cm × 31.25 cm) illuminated at 200 lux. The total distance traveled was recorded for 30 min by the VersaMax Animal Activity Monitoring System (Columbus, OH, USA). For melatonin treatment, there were n = 12 mice per group. For wheel treatment, there were n = 13–20 mice per group.