Pharmacological Research 117 (2017) 46–53

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Paradoxical sleep deprivation in rats causes a selective reduction in the expression of type-2 metabotropic glutamate receptors in the hippocampus

Isabella Panaccione[a], Luisa Iacovelli[b], Luigi di Nuzzo[b,c], Francesca Nardecchia[b,d], Gianluca Mauro[c], Delfina Janiri[a], Antonio De Blasi[e], Gabriele Sani[a], Ferdinando Nicoletti[b,f,1], Rosamaria Orlando[b,*,1]

[a] Unit of Psychiatry, Department of Neurosciences, Mental Health and Sensory Organs (Ne.S.M.O.S.), Faculty of Medicine and Psychology, Sapienza University of Rome, Via di Grottarossa 1035, 00189 Rome, Italy
[b] Department of Physiology and Pharmacology, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy
[c] Institute of Psychiatry and Psychology, Catholic University of the Sacred Heart, Largo F. Vito 1, 00168 Rome, Italy
[d] Department of Paediatrics, Child Neurology and Psychiatry, Sapienza University of Rome, Via dei Sabelli 108, 00185 Rome, Italy
[e] Department of Molecular Medicine, Sapienza University of Rome, Viale Regina Elena 291, 00161 Rome, Italy
[f] IRCCS Neuromed, Località Camerelle, 86077 Pozzilli (IS), Italy

ARTICLE INFO

Article history:
Received 13 June 2016
Received in revised form
10 November 2016
Accepted 22 November 2016
Available online 23 November 2016

Keywords:
Paradoxical sleep deprivation
mGlu2 receptor
Motor activity
BINA

ABSTRACT

Paradoxical sleep deprivation in rats is considered as an experimental animal model of mania endowed with face, construct, and pharmacological validity. We induced paradoxical sleep deprivation by placing rats onto a small platform surrounded by water. This procedure caused the animal to fall in the water at the onset of REM phase of sleep. Control rats were either placed onto a larger platform (which allowed them to sleep) or maintained in their home cage. Sleep deprived rats showed a substantial reduction in type-2 metabotropic glutamate (mGlu2) receptors mRNA and protein levels in the hippocampus, but not in the prefrontal cortex or corpus striatum, as compared to both groups of control rats. No changes in the expression of mGlu3 receptor mRNA levels or mGlu1α and mGlu5 receptor protein levels were found with exception of an increase in mGlu1α receptor levels in the striatum of SD rats. Moving from these findings we treated SD and control rats with the selective mGlu2 receptor enhancer, BINA (30 mg/kg, i.p.). SD rats were also treated with sodium valproate (300 mg/kg, i.p.) as an active comparator. Both BINA and sodium valproate were effective in reversing the manic-like phenotype evaluated in an open field arena in SD rats. BINA treatment had no effect on motor activity in control rats, suggesting that our findings were not biased by a non-specific motor-lowering activity of BINA. These findings suggest that changes in the expression of mGlu2 receptors may be associated with the enhanced motor activity observed with mania.

1. Introduction

Changes in the expression of group-II metabotropic glutamate receptor subtypes (mGlu2 and mGlu3 receptors) are consistently reported in the brain of mice and rats modeling psychiatric disorders, such as depression and schizophrenia. Mice with a low resilience to stress show a reduced expression of mGlu2 receptors in the hippocampus [1,2], and prenatal stressful events, which induce a psychotic-like phenotype, cause an epigenetic down-regulation of mGlu2 receptors in the mouse frontal cortex [3–6]. Low expression levels of mGlu2 receptors are also found in inbred strains of rats characterized by high levels of impulsivity [7]. Mice with genetic deletion of mGlu3 receptors show a deficit in working memory and an abnormal behavioral response to MK-801 [8]. In humans, polymorphic variants of the mGlu3 receptor gene have been associated with schizophrenia [9–18], and alterations in mGlu3 receptor dimerization have been reported in the prefrontal cortex of patients affected by schizophrenia [19].

An association between mGlu3 receptor expression or function and bipolar disorder (BP) is suggested by genetic studies in humans [20–22,18]. To our knowledge, there are no studies examining mGlu2 or mGlu3 receptor expression in animal models of BD

* Corresponding author at: Department of Physiology and Pharmacology, Sapienza, University of Rome, 00185 Rome, Italy.
E-mail address: rosamariaorlando@hotmail.com (R. Orlando).
[1] Co-last authors.

http://dx.doi.org/10.1016/j.phrs.2016.11.029
1043-6618/

or mania (the clinical hallmark of BD). Our selection of the most appropriate animal model moved from the clinical evidence that a decreased need for sleep is one of the most characteristic features of mania [23,24] and that sleep deprivation is a triggering factor for manic episodes, and can induce switches from depression to mania in patients affected by BD [25,26]. Paradoxical sleep deprivation in animals induces manic-like behaviors, such as insomnia and motor hyperactivity, which are corrected by lithium treatment [27,28]. Hence, paradoxical sleep deprivation has face, construct, and predictive validity as model of manic episodes. While the study of how mGlu receptors regulate sleep architecture has been the subject of extensive investigation [29–36], only two studies examined the effect of sleep deprivation on mGlu receptor expression. Positron emission tomography (PET) analysis with the mGlu5 receptor ligand, ^{11}C-ABP688, showed an increased mGlu5 receptor binding in limbic regions of healthy subjects after 33 h of controlled wakefulness [37]. In rats, sleep deprivation caused an increased expression of mGlu1α receptors and an increased dimerization between mGlu1α and either type-1 or type-2 $GABA_B$ receptors in the hippocampal CA1 region [38]. Information on how paradoxical sleep deprivation affects the expression of mGlu2 and mGlu3 receptors is lacking. We now report that paradoxical sleep deprivation in rats causes a selective down-regulation of mGlu2 receptors in the hippocampus, and that treatment with a drug that amplifies mGlu2 receptor function corrects the manic-like phenotype observed after sleep deprivation.

2. Methods and materials

2.1. Animals

We used male Sprague-Dawley rats (Charles River, Milan, Italy) of six weeks, an age that corresponds to mid-adolescence [39], ranging in weight from 150 to 180 gr. They were housed two or three per polycarbonate rat cage under a 12 h light/dark cycle (light on at 7:00 a.m.) with free access to food and water. Rats were allowed to acclimate for one week prior to experiments, and were gently handled every day for three days before the sleep deprivation procedure to minimize stress effects. Animal experiments were performed in full compliance with the ARRIVE guidelines [40]. All efforts were made to minimize the number of animals used and their suffering. All experimental procedures were carried out in strict compliance with the Italian (D.L. 26/2014) and European Union (2010/63/EU) guidelines of animal care and experimentation.

2.2. Drugs and treatments

Biphenyl-indanone A (BINA) (Cayman Chemical, Ann Harbour, USA) was dissolved in saline containing 5% dimethyl sulfoxide (DMSO), and injected i.p. at the dose of 30 mg/kg. Valproic acid sodium salt (VPA; Depakin, Sanofi), was also dissolved into saline containing 5% DMSO, and injected i.p. at the dose of 300 mg/kg.

2.3. Paradoxical sleep deprivation

Paradoxical sleep deprivation was performed by using the "single platform" technique described by Gessa et al. [27] with the difference that rats were exposed to the procedure for 48 instead of 72 h to limit the stress inherent to the model as much as possible. Animals were placed for 48 h (starting at 9:00 a.m.) into a Plexiglas tank (35 × 27 × 26 cm), open on top, containing a glass cylinder 12 cm tall and 7 cm diameter (small platform). The upper surface of the cylinder was the only support for the animal when the tank was filled with warm water kept at a constant temperature of 27 °C. When animals entered the paradoxical phase of sleep, they fell into the water because of muscle atonia, thereby interrupting the rapid eye movement (REM) phase. We included a stress control group subjected to the same procedure of isolation and immobility, except that a larger platform was used (12 cm tall and 12 cm diameter), allowing the animals to remain on the platform during the REM phase of sleep. Food and water were made available through a grid placed on top of the water tank. Unstressed control rats remained in their home cage.

2.4. Experimental design

We performed three independent experiments. In the first experiment, 23 rats were divided into three groups (7–8 rats per group): (i) home-caged unstressed control (Ctrl) rats; (ii) isolation-stress control rats positioned on the large platform (LP), that allowed rats to sleep without falling into the water; and, (iii) sleep-deprived (SD) rats, positioned on the small platform that caused the animal to fall into the water at the onset of the REM phase of sleep. We assessed locomotor activity in an open field arena in 6 animals per group immediately after the end of the SD session. All animals were used for biochemical determinations of mGlu receptor expression at the end of the assessment of locomotor activity. In the second experiment, two groups of "home-caged" controls and two groups of SD rats (n = 5–6 per group) were treated i.p. with either BINA or vehicle (saline containing 5% DMSO). An additional group of SD rats was treated with VPA as an active comparator. To reduce the number of animals to its minimal, control rats were not treated with VPA because acute injection of VPA at the doses used here was shown not to affect locomotor activity and rearing in rats [41]. BINA, VPA, or vehicle were injected only once 60 min prior to the termination of the SD procedure (or the equivalent time in home-caged controls), and rats were tested for locomotor activity in the open field apparatus immediately after the end of the SD session. In the third experiment, two groups of "home-caged" controls and two groups of SD rats (n = 6 per group) were treated i.p. with either BINA or vehicle (saline containing 5% DMSO), as in experiment 2, and tested in the Elevated Plus Maze (EPM) followed by Social Interaction test. This analysis was performed 2 h after the SD session to limit the influence of post-SD on motor activity. In experiment 3, vehicle or BINA were injected 60 min after the SD session (i.e., 60 min prior to the EPM testing). Animals from the experiments 2 and 3 were exclusively used for behavioral analysis.

2.5. Behavioral analysis

2.5.1. Locomotor activity

Locomotor activity was evaluated in an open field arena (45 × 30 cm) immediately after the termination of the SD procedure. The relatively small size of the arena was choosen to facilitate the assessment of the locomotor and exploratory activity [42]. The test was performed under normal cool room light. Each animal was videotaped, and locomotor activity was evaluated for 30 min by an experienced observer, who was not aware of the experimental condition ("home cage", small platform, large platform, or drug treatments). The floor of the arena was divided into 6 squares (15 × 15 cm) labeled in black, and a central square (15 × 15 cm) overimposed to the central portion of the other squares and labeled in red. The times each rat crossed the separation lines with all four paws ("number of crossing") [43], the time spent in the central square, and the episodes of rearing (exploratory vertical activity) were recorded. Latency to immobility was determined as the first occurrence of <5 line crossings during a period of at least 5 min [44]. Between each trial, the open field arena was wiped clean with 10% ethanol.