Behavioural Brain Research 311 (2016) 287–297

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Off-target potential of AMN082 on sleep EEG and related physiological variables: Evidence from mGluR7 (−/−) mice

A. Ahnaou*, L. Raeyemaekers, H. Huysmans, W.H.I.M. Drinkenburg

Dept. of Neuroscience Discovery, Janssen Research & Development, A Division of Janssen Pharmaceutica NV. Turnhoutseweg 30, B-2340 Beerse, Belgium

HIGHLIGHTS

- AMN082 is widely used to explore the functional role of mGluR7.
- AMN082 increased state transitions towards wakefulness and reduced slow wave activity during sleep in rats.
- MGluR7 (−/−) mice exhibit comparable spontaneous sleep wake states as WT littermates.
- AMN082 produced similar arousal and hypothermia pattern in mGluR7 (−/−) and WT mice.
- AMN082 elicited an off-target action that contributes to its waking properties.

ARTICLE INFO

Article history:
Received 11 December 2015
Received in revised form 27 April 2016
Accepted 16 May 2016
Available online 17 May 2016

Keywords:
Glutamate
mGluR7
AMN082
Rats
mGluR7 (−/−) mice
Off-target
Sleep-wake cycle
EEG oscillations
Body temperature
Locomotor activity
Psychiatric and neurodegenerative disorders

ABSTRACT

The metabotropic glutamate receptor type 7 (mGluR7), a most abundant presynaptic G protein-coupled receptor in the brain provides an attractive mechanism to fast fine-tune abnormal excitatory neurotransmission and synaptic plasticity associated with emotional and cognitive impairments in neuropsychiatric and neurodegenerative disorders. Preclinical studies using AMN082, the mGluR7 allosteric agonist, produced conflicting results, so that results of further in-vivo studies are needed. Here, we investigated effects of subcutaneous administration at the lights onset of AMN082 on sleep-wake architecture and spectral contents in rats. In an attempt to solve the reported mixed results, we estimated the specific functional effects in mGluR7 (−/−) mice and their wild type (WT) littermates. In rats, AMN082 (2.5 mg/kg) elicited a primary waking effect over the first 2 h post-administration by consistent increases in the number of waking bouts and transitions from sleep states towards wakefulness. In mice, baseline recordings over 72 h showed comparable spontaneous sleep-wake cycle in mGluR7 (−/−) mice and their WT littermates, suggesting that mGluR7 is not involved in the regulation of vigilance states. Remarkably, cortical arousal properties of AMN082 were confirmed in WT mice, and occurred concomitantly with a marked decrease in body temperature, likely dissociated from locomotor activity. Surprisingly, the wake arousal and hypothermia effects of AMN082 were also observed in mGluR7 (−/−) mice. AMN082 significantly attenuated the slow wave activity during sleep and had no effect on waking EEG power in the 4–50 Hz range. The present findings in rats do not lend support to proposed somnogenic effects of AMN082, while comparable responses in WT and mGluR7 (−/−) mice provide further evidence of an off-target action of AMN082 that contributes to its waking properties.

1. Introduction

The brain glutamatergic system is critically involved in the regulation of several basic central nervous system (CNS) functions including sleep-wake, attention and memory, metabolic and emotional homeostasis via activation of a family of ligand-gated inotropic glutamate (NMDA, AMPA and kainate) and a family of G protein-coupled receptors (mGluR) [1–3]. Of the eight mGluRs subtypes, mGluR7 is widely distributed at presynaptic clefts in key brain regions associated with emotion and learning such as the cingulate cortex, frontal cortex, amygdala, hippocampus and locus coeruleus [4–6]. As a presynaptic autoreceptor, it is inactive during normal neurotransmission and becomes active during times of excessive glutamate neurotransmission in order to inhibit further neuronal glutamate release [7–9]. Therefore, the mGluR7 provides an attractive mechanism to fast fine-tune abnormal synaptic responses associated with cognitive impairments in a range of psychiatric and neurological disorders.

* Corresponding author.
E-mail address: aahnaou@its.jnj.com (A. Ahnaou).

http://dx.doi.org/10.1016/j.bbr.2016.05.035
0166-4328/

The generation of mGluR7 knock-out (−/−) mice and the development of selective pharmacological agents at the mGluR7 have given important insights into the potential role of this receptor in multiple conditions including drug addiction, depression, anxiety, schizophrenia, pain and Parkinson and Alzheimer's diseases [10–12]. Mice lacking mGluR7 displayed deficits in short term working memory in the arm maze tasks [13], as well as an impaired contextual fear response [14,15]. The anxiolytic phenotype of mGluR7 (−/−) was found in multiple anxiety tests such as the elevated plus maze (EPM), light–dark box, staircase, open field and marble burying and the stress-induced hyperthermia [10,16,17]. The antidepressant-like phenotype of mGluR7 (−/−) mice was observed in the forced swim and tail suspension tests [10,18]. The highly selective and systemically potent mGluR7 agonist (*N*,*N*0-dibenzhydrylethane-1,2-diamine dihydrochloride) (AMN082) elicited anxiolytic and antidepressant effects in wild type but not in mGluR7 (−/−) mice [17]; as well as reduced the activity of the hypothalamo-pituitary adrenal stress axis [19].

However, pharmacological studies using AMN082 have yielded contradictory results as to those described in earlier phenotypic studies in mGluR7 (−/−) mice pointing to a role of mGluR7 in anxiety and depression. Accordingly, mGluR7 (−/−) mice exhibited anxiolytic and antidepressant phenotypes, while activation of the receptor was expected to elicit an opposite effect. However, AMN082 produced an anxiolytic-like effect in the stress-induced hyperthermia and four plate tests [17] as well as an antidepressant effect in the forced swim and tail suspension tests [20]. Therefore, the central specificity of AMN082and its use in behavioral pharmacology remains questionable.

Recently, AMN082 has been reported to increase total sleep time, which pointed towards a role of the mGluR7 in the regulation of sleep-wake cycle [21]. However in the later study larger doses of AMN082 were used and recordings were performed over a short period of 3 h. The present study aimed at profiling effects of AMN082 on sleep-wake architecture in rats over a longer period, to evaluate the immediate and possible long term effect across the light dark cycle. In addition, we have used the mGluR7 (−/−) mice to investigate the specificity of its central effects and to clarify the potential "off-target" stimulating profile of AMN082 as assessed by sleep-wake behavior and related physiological variables.

2. Material and methods

2.1. Animals and surgery procedure

All experimental protocols were carried out in strict accordance with guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC), and of the European Communities Council Directive of 24th November 1986 (86/609/EEC) and were approved by the ethical committee of Janssen Research and Development. Male Sprague Dawley rats (Charles River, France; n = 32) weighing 250–300 g and male mGluR7 (−/−) mice with their WT weighing 25–30 g (kindly provided by Dr. Rudi D'Hooge, University of Leuven, Belgium) were used in the polygraphic experiments. The genotype was confirmed by PCR analysis of tail DNA. All animals were housed in individually ventilated cages, located in a sound-attenuated chamber, and were maintained under controlled environmental conditions throughout the study: 22 °C ± 2 °C ambient temperature, relative humidity 60%, standard 12:12 light cycle regime (illumination intensity: ∼100 lx) and had free access to standard laboratory food chow and tap water.

Surgery in rats was performed using the protocol described earlier [22]. Under Isoflurane anaesthesia, rats were implanted with 4 fixing stainless steel screws for the recording of the frontal and parietal electroencephalogram (EEG). For the recording of the electro-oculogram (EOG) and electromyogram (EMG), stainless steel wires were placed in the peri-orbita and inserted into the nuchal muscle, respectively. Electrodes were fitted into an 8-hole connector and were fixed with dental cement to the cranium. After surgery, animals were given analgesics and allowed a recovery period before adaptation period to recording conditions. During the registration sessions, animals were connected with a rotating swivel to a bipolar recorder amplifier (Embla, MedCare Flaga, Iceland) for polygraphic signals recordings with an input range of ±500 mV.

Surgery in mice was performed under deep isoflurane anesthesia, (4% induction, and 1.5% l maintenance) and body temperature was thermostatically controlled using the heating pad, through the operation and following recovery. mGluR7(−/−) mice and their wild type (WT) littermates were instrumented with the telemetry transmitter TA10ETA-F20 (Data Science International, USA), containing biopotential electrodes for monitoring the electroencephalographic (AP −2.3 mm, L ± 1.4 mm from bregma), according to the procedure used in an earlier study [23].

After surgery animals were given 0.3 ml analgesic (Carprofen, Rimadyl, 50 mg/ml, Pfizer Ltd., UK diluted 1:10 s) and local analgesic was applied on the wounds (Lidocaine spray Xylocaine, 1% solution, Astra Pharmaceuticals Ltd., UK). Then, animals were individually placed in their home cage, kept warm in a heating box set at 26 °C ± 2 °C to avoid hypothermia and this temperature was progressively decreased over a 5 days to reach room temperature. The animals were allowed to recover from surgery for at least 2 weeks.

2.2. Experimental design, recording and analysis

After two weeks recovery and adaptation to the recording conditions in rats, the EEG, EOG, EMG signals and body movement activities were monitored for 20 h; the first recording session started at end of the second hour of the light phase of the 12 light/12 dark cycle and lasted 20 h after the administration of saline in all animals. The following recordings were performed for the same duration and circadian conditions following the administration of vehicle and different doses of AMN082 (n = 32, 8 subjects for each condition). The online acquisition of EEG/EOG/EMG was performed using a bipolar recorder system (Embla, Medcare, Iceland), amplified and digitized at sample rate of 200 Hz. Treatment of the signals was performed by a software package (Somnologica, MedCare, Iceland), which turns the computer into a polygraphic workstation for signal recording. The EEG signals were high-pass filtered with 0.5 Hz filter and Notch filter of 50 Hz was used to discard alternate current contamination.

Sleep polysomnographic variables were determined offline as described elsewhere [22] using a sleep stages analyzer, scoring each 2-s epoch before averaging stages over 30-min periods. Sleep-wake states classification were assigned based upon combination of dynamics of 5 EEG frequency domains, integrated EMG, EOG, and body activity level: active wake; passive wake; intermediate stage; light sleep; deep sleep and rapid eye movement (REM) sleep. Different sleep-wake parameters were determined for 20 h after administration of drugs or vehicle: amount of time spent in each vigilance state, sleep parameters, latencies to sleep states onset, sleep period and the number of transitions between states.

Changes in EEG spectral power were determined in the active waking and deep sleep EEG artefact free epochs of 2 s over the first 4 h post-administration during the first 24 h baseline and the second 24 pharmacological conditions. Fast Fourier Transform analysis was used to compute the EEG power (V^2) in 0.5 Hz increments within the EEG frequencies ranging from 1 to 50.0 Hz in consecutive artefact-free 2 s epochs frequency range of 0.5–50 Hz and data were collapsed in 1 Hz bins during the first 4 h post-treatment. Spectral power profiles were expressed as the mean of absolute spectral pro-