Neuroscience Letters 463 (2009) 249–253

Contents lists available at ScienceDirect

Neuroscience Letters

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

Cholinergic stimulation of the nucleus basalis of Meynert and reticular thalamic nucleus affects spike-and-wave discharges in WAG/Rij rats

Rustam K. Berdiev[a,*], Gilles van Luijtelaar[b]

[a] Lomonosov Moscow State University, Faculty of Biology, Department of Human & Animal Physiology, Leninskie Gory, 1-12, Moscow 119992, Russia
[b] Donders Center for Cognition, Radboud University Nijmegen, Nijmegen, The Netherlands

ARTICLE INFO

Article history:
Received 16 March 2009
Received in revised form 16 July 2009
Accepted 22 July 2009

Keywords:
Absence epilepsy
WAG/Rij rat
Nucleus basalis
Reticular thalamic nucleus
Carbachol
Scopolamine

ABSTRACT

The role of the cholinergic innervated nucleus basalis of Meynert (NB) and reticular thalamic nucleus (RT) in the generation or modulation of spontaneously occurring spike-and-wave discharges (SWDs) was investigated in the WAG/Rij rat model of absence epilepsy. The cholinergic agonist carbachol and the muscarinic antagonist scopolamine were injected in the NB and RT in the doses of 0.55 and 5.5 nmol while the EEG was recorded. Carbachol injected in the NB decreased the number and the mean duration of SWDs. Scopolamine alone had no influence on SWDs, but could antagonize the effects of carbachol if administered simultaneously in NB. Injections of carbachol in the RT inhibited the occurrence of SWDs, but did not affect the mean duration. Scopolamine administered in the RT had no influence on seizure activity. It is concluded that cholinergic stimulation of the NB or the RT inhibits the cortical synchronous activity characterizing SWDs.

Absence epilepsy is characterized by transitory decrease of consciousness accompanied by generalized bilateral spontaneous spike-and-wave discharges (SWDs) in the electroencephalogram (EEG) with frequency of 2.5–4 Hz in humans [3] and 5–11 Hz in several genetic rodent models [27,15,25]. The corticothalamic circuitry plays a leading role in the occurrence of absence seizures in WAG/Rij and GAERS rat models [17,21]. The frontoparietal cortex, the reticular thalamic nucleus (RT) and the ventrobasal complex of the thalamus strongly interconnected through a cortical-thalamocortical network are thought to be crucial structures in initiating, propagation and maintenance of SWDs [2,6].

It is evident that the mechanisms controlling sleep and wakefulness are involved in the regulation of absence seizures since absence paroxysms occur predominantly during passive wakefulness, drowsiness and light slow-wave sleep [19,7,15]. The basal forebrain cholinergic system plays an important role in controlling the arousal level of the brain.

The nucleus basalis of Meynert (NB) plays a pivotal role in modulating cortical activity and maintenance of arousal and sleep states. The NB contains a population of large cholinergic neurons distributed throughout the ventral pallidum and substantia innominata. These neurons provide a major, topographically organized cholinergic input to the entire neocortex [24]. Cholinergic and GABAergic neurons from the NB provide excitatory and inhibitory inputs to the reticular thalamus nucleus (RT) [1,10]. Thus, NB cholinergic neurons can modulate intrinsic cortical responsiveness both by direct input to the neocortex and indirect input via the thalamus. It has been found, that bilateral electrolytic lesions of the NB increased spontaneous high-voltage spindle activity in rats [6]. Others reported that a complete lesion of the NB with ibotenate and quisqualate irreversibly suppressed the occurrence of SWDs in GAERS [9], while a selective lesion of cholinergic neurons by 192 IgG-saporin showed opposite effect in WAG/Rij rats [4]. In order to confirm the assumption that this enhancement of SWDs is closely related to the loss of cholinergic NB neurons, a pharmacological approach was followed. It is hypothesized that local administration of the cholinergic agonist carbachol in the NB will decrease SWD activity in WAG/Rij rats.

The RT receives afferent cholinergic projections from the NB [14]; it is a population of GABAergic neurons, composing a shell-shaped sheet at the lateral margins of the thalamus [12]. Inhibitory circuits arise in the RT and are important in producing synchrony between neurons in the thalamocortical network [26]. The afferents to the RT from thalamus and cortex, together with those from the lateral dorsal tegmental nucleus and pedunculopontine tegmental nucleus of the brainstem and NB [10] play a crucial role in the control of firing patterns of thalamocortical relay cells, which can be in "tonic" or "burst" firing modes. The last one has been observed during sleep and epileptic discharges of the absence type [26]. All cholinergic synapses are presynaptic to dendrites of the RT [10]. It has been shown that stimulation of M2 ACh receptors in the RT suppresses neocortical high-voltage spindles (similar characteristics as

* Corresponding author. Tel.: +7 495 939 27 92.
E-mail address: rberdiev@gmail.com (R.K. Berdiev).

0304-3940/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2009.07.068

SWDs) in aged Wistar rats [22]. Here we wanted to confirm whether the role of cholinergic system in the RT as was established in old Wistar rats is the same in this genetic model for human absence epilepsy (WAG/Rij). Carbachol and scopolamine were injected in the RT and it was investigated whether the RT is able to maintain SWDs under the influence of cholinergic stimulation.

The Ethical Committee on Animal Experimentation of Radboud University Nijmegen approved the experimental protocol used in this study; it was also in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). 10-Month-old male WAG/Rij rats 360–450 g ($n = 22$) were used as subjects. The animals were housed singly in macrolon cages with food and water *ad libitum*. A 12-h light/dark cycle (light off from 08:00 to 20:00) was maintained during the experiment.

Rats were anaesthetized with Isoflurane (2.5%) and placed in a stereotaxic frame (David KOPF Instruments). A tripolar EEG electrode set (MS 333/2A, Plastics One) was placed unilaterally: recording electrode was placed on the frontal cortex (AP +2 mm, ML −3.5 mm), reference electrode on the occipital cortex (AP +6 mm, ML −4 mm) and ground electrode over the cerebellum. A guide cannula (C312G, Plastics One) was unilaterally implanted above the NB (AP −1.5 mm, ML −3 mm) ($n = 11$) or RT (AP −1.4 mm, ML −2 mm) ($n = 11$) in the right hemisphere. Guide cannulas were 2 mm shorter than the depth of an aimed brain structure. Stereotaxic coordinates were chosen according to the atlas of Paxinos and Watson [20] with bregma as a reference point. Following surgery, the rats were allowed to recover for at least 14 days.

Before the recording the rats were moved to individual recording cages and adapted to the recording conditions overnight. The EEG was continuously recorded in freely moving rats for 1 h before and 2 h after each injection, always at the same time of day (10:00–13:00). The data acquisition system was WINDAQ Software (Dataq Instruments). EEG was amplified and filtered between 1 and 100 Hz, the sampling frequency was 512 Hz.

The number, total duration of SWDs over 20 min and 1 h time periods as well as mean duration of SWDs were quantified using commonly used criteria [27]. Representative power spectra of the EEG were obtained by averaging five 4-s spectra in periods of 20 s before and after drug administration during the periods of passive wakefulness. Numerical values were obtained for the delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz) and beta (12–30 Hz) bands using Spectranes software (ver. 2.00a, UMC St. Radboud, Dept. of Anesthesiology, Nijmegen, the Netherlands).

Carbamylcholine chloride (carbachol), that acts as both muscarinic and nicotinic ACh receptors agonist, and the competitive antagonist at muscarinic receptors scopolamine hydrochloride (scopolamine) were purchased from Sigma–Aldrich, the Netherlands. For each drug doses of 0.55 and 5.5 nmol were used. Intraparenchymal infusions were made through 1-μl Hamilton syringes connected to the manual pump. Injection needle was connected to the syringe by a thin flexible tube. Before the injection the tube was filled with distilled water and afterwards the drug solution was placed into the tip of the needle. The drugs were injected in NB or RT in a final volume of 0.5 μl at a rate of 0.1 μl/min at a depth of 8 and 6.8 mm from the skull surface respectively. The animals were injected in NB or RT with carbachol, scopolamine and a combination of both drugs (carbachol plus scopolamine). In case of the combination of both drugs, they were injected in the same dose of 5.5 nmol in a volume of 0.25 μl, and scopolamine was injected fist. During the experiment each animal received totally 6 injections. As a control solution, 0.5 μl of saline was injected. In half of the animals, randomly chosen, the drug was given first, and was followed by the control saline solution after a washout period of minimally 48 h, and the reverse procedure was used for the other animals. The order of the doses was also reversed in half of the animals. On purpose to check whether the factor order was crucial and whether our solution was good enough, we compared baseline measurements before each subsequent injection. There were no significant differences between the various baseline measurements or an interaction with order.

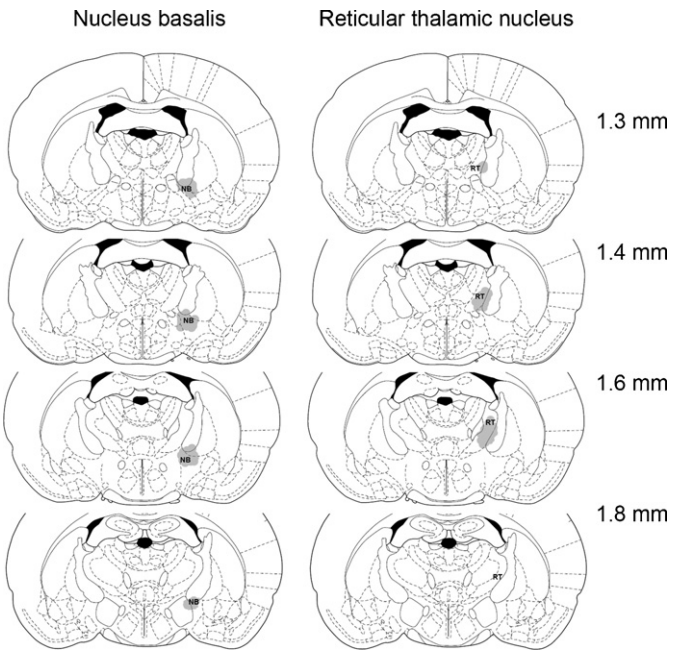

Fig. 1. Localization of unilateral injection sites (marked grey) of carbachol and scopolamine in the NB and RT, according to Paxinos and Watson rat brain atlas [20]. NB, Nucleus basalis of Meynert; RT, reticular thalamic nucleus.

The behaviour of the rats was closely observed for 40 min after the injection from an adjacent room through a window and encoded on the disk to measure/determine quantitative data (number of times of occurrence and duration of the following behavioural categories) on locomotor (active), automatic and passive behaviour. The data were analyzed with The Observer (Noldus, Wageningen, the Netherlands).

After the pharmacological study the animals were euthanized with a Nembutal injection (SANOFI SANTE B.V., France, 60 mg/kg) and perfused through the heart with saline and afterwards with 4% buffered paraformaldehyde (pH 7.3). The brains were removed and cryoprotected in 30% sucrose for 3 days. 40 μm sections were cut on a cryomicrotome and stained with cresyl violet in order to determine the sites of the injections. Only animals with accurate cannula placement were included in the analysis (Fig. 1).

Experimental variables were analyzed using repeated-measures ANOVA, with time and dose as within and between subjects factors respectively, followed by LSD test for post hoc comparisons, and Wilcoxon matched-pairs test. The values are given as means ± SE. A probability of $p < 0.05$ was considered significant.

Unilateral injections of carbachol in the NB significantly decreased the occurrence and duration of SWDs in WAG/Rij rats. Detailed analyses of SWDs pattern (every 20 min) revealed a reversible inhibitory effect of carbachol ($F = 5.71$, df 2,29, $p < 0.01$). Post hoc tests showed the significant decrease of the number of SWDs after carbachol administration in a dose of 0.55 nmol during the first 20 min after the injection, and after a dose of 5.5 nmol during 40 min, compared with the same values after saline administration (Fig. 2A). The effect was dose-dependent for the first 20 min. No SWDs have been observed during the first 20 min after 5.5 nmol of carbachol infused in the NB, with an exception of one animal among the group of eight and only four animals showed several SWDs 40 min after the injection (Fig. 2D). Also, the mean duration of SWDs was decreased for 40 min after injection of 5.5 nmol of carba-