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Role of L- and T-Type Calcium Channels in Regulation of Absence Seizures in Wag/Rij Rats

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In chronic experiments on five groups of WAG/Rij rats (a genetic model of absence epilepsy; six animals in each group), we recorded EEG activity from the S1po cortical area through implanted electrodes and subjected the cortex to the action of four agents affecting L- and T-type calcium channels (injections through an implanted cannula). A blocker of L-type channels, verapamil hydrochloride, an agonist of these channels, Bay K8644, an antagonist of T-type calcium channels, L-ascorbate, and an agonist of the latter channels, PMA, were used. The parameters of 7- to 10-Hz spike-wave discharges, SWDs, spontaneously generated in the cortex of this rat strain (frequency within SWDs, mean duration of the latter, and their number) were measured within the baseline interval (before injections) and within three subsequent 20-min-long post-injection intervals. Normal saline was injected in the control group. There were no significant differences in the mean peak frequency in SWDs between all examined groups ($P > 0.05$ in all cases). Verapamil significantly (by more than 40%; $P < 0.05$) decreased the mean SWD duration throughout the entire period of post-injection observation. The dynamics of the Bay K8644 effects were rather similar, but the intensity of SWD duration changes was somewhat smaller. Both the above agents in the doses used dramatically decreased the number (frequency of appearance) of SWDs within the observation period. L-ascorbate also suppressed SWD generation. The duration of these phenomena decreased mildly, while their number dropped dramatically. In the PMA group, the number of SWDs increased significantly (by 50%, $P < 0.05$) within the first 20-min-long interval, but this was not observed within subsequent intervals. These findings confirm that blocking or activating of L- and T-type Ca^{2+} channels in the S1po area (cortical focus area) can significantly control generation of SWDs during absence seizures. Possible mechanisms underlying actions of the tested agents are discussed.

Keywords: absence seizures, spike-wave discharges, WAG/Rij rats, L- and T-type calcium channels.

INTRODUCTION

Absence seizures are believed to be a phenomenon typical of generalized non-convulsive epilepsies. These events are characterized by some general features, like brief unresponsiveness to environmental stimuli and interruption of consciousness. Absence seizures are characterized by symmetric, bilateral, and synchronous 3-Hz spike-wave discharges (SWDs) [1]. In absence epilepsy, abnormal motor activities may not be recognized [2]. The annual incidence rate of absence epilepsy has been estimated to be about 1.2-7.1 per 100,000 in different populations [3].

Wistar Albino Glaxo Rijswijk (WAG\Rij) rats are described to be a genetic model for absence epilepsy; these animals exhibit 7- to 10-Hz SWDs lasting from 1 to 30 sec [4, 5]. SWDs are not generated during active wakefulness and REM sleep, but they occur in wakefulness with a low level of alertness [6]. Sixteen to twenty discharges per hour are usually recorded [7]. During seizures, WAG/Rij rats remain immobile and twitch their vibrissae and facial muscles [8]. In line with the cortical focus theory and nonlinear association analysis of SWDs, the S1po is the initiation site for SWDs. Seizure activities that were produced in this zone disseminated quickly in other areas of the cortex and thalamus. It was found that injection of ethosuximide into the ventrobasal

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thalamus and RTN demonstrated a low efficacy in suppression of SWDs [9, 10].

There is strong evidence that entry of Ca^{2+} ions into neurons plays a significant role in generation of seizures [11]. Throughout epileptiform activity, intracellular Ca^{2+} concentration increases, while extracellular Ca^{2+} concentration is reduced [12]. High- and low-voltage-activated (HVA and LVA) calcium channels are involved in the regulation of neuronal excitability and production of absence SWDs. High-voltage-activated calcium channels include different subunits (α, β, γ, and σ). In mammals, genome expression forms three large families of subtype β1. Four different kinds of L-type HVA calcium channels belong to the Cav1 family (Cav1 through Cav1.4) [13], Cav1.2, and Cav1.3 channels are located on neuronal membranes, generally on the dendrites [14]. In hippocampal neurons, calcium currents in cells depend mostly on the presence of L-type channels [15].

Low-voltage-activated T-type channels are monomers of subunit β_1, and their activation results from relatively small membrane depolarization. Such channels are members of the Ca_v3 family, and they are divided into three groups ($Ca_v3.1$-$Ca_v3.3$) [13]. T-type calcium channels play key roles in the regulation of neuronal excitability. Hyperactivity of these channels causes some neurological disorders, such as absence seizures and neuropathic pain [16]. It was mentioned that the activity of T-type channels is increased in a mouse model of epilepsy [17].

In our study, we examined the role of L- and T-type calcium channels on SWDs recorded from the cortical S1po area in WAG/Rij rats. The experiment addressed an important question of how SWDs can be changed after injections of agents that effectively influence the above-mentioned channels. These are a blocker of L-type calcium channels, verapamil hydrochloride; an L-type channel agonist, Bay K8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate), an antagonist of T-type calcium channels, L-ascorbate; and T-type channel agonist, PMA (phorbol 12-myristate 13-acetate, a protein kinase C activator).

METHODS

Animals and Drugs. Experiments were carried out on 30 WAG/Rij rats (age from 4 to 6 months, body mass from 220 to 260 g). Animals were maintained under standard lab conditions (22 °C, light/dark cycle 12/12 h, and unlimited access to food and water until the presupposed time of the experiment).

Bay K8644, verapamil, and PMA were purchased from Tocris (Great Britain), while L-ascorbate was purchased from Sigma (USA). Verapamil and L-ascorbate were dissolved in 0.9% saline. For injections into the S1po cortex (in the volume of 1 µl), Bay K8644 was dissolved in 0.9% saline and ethanol. To inject 500 nmol PMA, a stock solution containing 2 mM PMA in DMSO was dissolved in saline to the necessary concentration.

Surgical Procedure. Rats were anesthetized i.p. by ketamine (80 mg/kg) and xylazine (5 mg/kg) [18]. All animals were implanted with two cortical stainless steel electrodes for EEG recording and a cannula for drug injection. The coordinates of the cannula tip were the following: 2.1 mm posterior, 5.5 mm lateral to the bregma, and 4.0 mm vertical from the skull surface (according to the stereotaxic atlas [19]).

The monopolar EEG recording electrode was in the frontal cortical region of the right hemisphere (coordinates: AP = 0.22, L = 0.24, and V = 0.26), while the reference electrode was on the occipital cortex. Electrodes were fixed in the sockets by pins. The cannula socket was fixed to the skull by dental cement.

Recording and Injection. After 1-week recovery from surgery, rats were settled in a Faraday cage, and EEG was recorded from freely moving animals. Spike-wave discharges (Fig. 1) were recorded 20 min before and three times within 20-min-long intervals after drug injections. Mild natural stimuli (moderate sound or touch) were applied to prevent the animal from sleeping. Separate groups of rats were used to characterize the effects of verapamil, Bay K8644, L-ascorbate, and PMA. For bilateral injection of 1 µl of the solution, a 27 G cannula needle in the guide cannula was connected to a 10 µl Hamilton syringe. A dose of 22.4 µmol of Bay K8644 was injected. The verapamil hydrochloride injection dose was 40 µmol. In addition, 500 nmol of PMA and 100 µmol of L-ascorbate were injected in the respective animal groups.

Statistical Analysis. For statistical analyses, SPSS13 software was used. Variables (mean within-SWD peak frequency, mean duration, and number of SWDs) were analyzed by intragroup repeated measures and one-way ANOVA between groups. The Sidok test was applied for *post-hoc* analysis.