Journal of the Neurological Sciences 375 (2017) 450–459

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

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

The antiepileptogenic effect of low-frequency stimulation on perforant path kindling involves changes in regulators of G-protein signaling in rat

Simin Namvar [a], Yaghoub Fathollahi [a], Mohammad Javan [a], Maryam Zeraati [a], Mohammad Mohammad-Zadeh [b], Amir Shojaei [a], Javad Mirnajafi-Zadeh [a,*]

[a] Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[b] Cellular and Molecular Research Center, Department of Physiology and Pharmacology, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran

ARTICLE INFO

Article history:
Received 2 July 2016
Received in revised form 27 January 2017
Accepted 20 February 2017
Available online 22 February 2017

Keywords:
Seizure
Brain stimulation
Regulators of G-protein signaling
Dentate gyrus

ABSTRACT

G-protein coupled receptors may have a role in mediating the antiepileptogenic effect of low-frequency stimulation (LFS) on kindling acquisition. This effect is accompanied by changes at the intracellular level of cAMP. In the present study, the effect of rolipram as a phosphodiesterase inhibitor on the antiepileptogenic effect of LFS was investigated. Meanwhile, the expression of α_s- and α_i-subunit of G proteins and regulators of G-protein signaling (RGS) proteins following LFS application was measured. Male Wistar rats were kindled by perforant path stimulation in a semi-rapid kindling manner (12 stimulations per day) during a period of 6 days. Application of LFS (0.1 ms pulse duration at 1 Hz, 200 pulses, 50–150 μA, 5 min after termination of daily kindling stimulations) to the perforant path retarded the kindling development and prevented the kindling-induced potentiation and kindling-induced changes in paired pulse indices in the dentate gyrus. Intra-cerebroventricular microinjection of rolipram (0.25 μM) partially prevented these LFS effects. Twenty-four hours after the last kindling stimulation, the dentate gyrus was removed and changes in protein expression were measured by Western blotting. There was no significant difference in the expression of α-subunit of G_s and $G_{i/o}$ proteins in different experimental groups. However, application of LFS during the kindling procedure decreased the expression RGS4 and RGS10 proteins (that reduce the activity of $G_{i/o}$) and prevented the kindling-induced decrease of RGS2 protein (which reduces the G_s activity). Therefore, it can be postulated that the $G_{i/o}$ protein signaling pathways may be involved in antiepileptogenetic effect of LFS, and this is why decreasing the cAMP metabolism by rolipram attenuates this effect of LFS.

1. Introduction

Epilepsy is a prevalent neurological disorder. Approximately 10% of people have at least one epileptic seizure during their lifetime, and a third of these will develop epilepsy [15]. Unfortunately, 30 to 40% of epileptic patients are resistant to current drug therapy [53,56,60]. Therefore, it is necessary to develop alternative therapeutic techniques. Deep brain stimulation (DBS) has been considered a possible therapy for epilepsy for >30 years. Recently, DBS has been approved by the U.S. Food and Drug Administration (FDA) [2], and now DBS is moving to the point of clinical utility, especially for drug-resistant epilepsies [18,19,40,42,43,47].

Mounting evidence shows that the application of deep brain stimulation in the form of low-frequency electrical stimulation (LFS) has therapeutic effects in both epileptic patients [3,35] and experimental models of epilepsy, including kindling [25,57,66]. Although brain stimulation has been used since ancient times for treatment of different brain disorders [58], its therapeutic mechanisms have not been completely understood. It has been suggested that the mechanisms involved in depotentiation or long-term depression may have a role in the anticonvulsant effects of LFS. However, there are few studies addressing the antiepileptogenic mechanisms of LFS when it is applied during kindling acquisition.

Our previous studies showed that activation of two G protein-coupled receptors (adenosine A_1 and galanin GalR1 and GalR2) may have a role in mediating the anticonvulsant action of LFS in perforant path kindling [33,46,55]. We reported that the antiepileptogenic effects of LFS on perforant path kindling are exerted through activation of adenosine A_1 receptors [33,46] and are accompanied with prevention of the kindling-induced increase in cAMP levels [46]. There is a strong relationship between cAMP concentration and seizure severity

Abbreviations: ADD, afterdischarge duration; cAMP, cyclic adenosine monophosphate; RGS, regulators of G-protein signaling; KLFS, kindled + LFS; LFS, low frequency stimulation; LTP, long term potentiation; pEPSP, population excitatory post-synaptic potential; PS, population spike.

* Corresponding author at: Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, PO Box: 14115-111, Iran.
E-mail address: mirnajaf@modares.ac.ir (J. Mirnajafi-Zadeh).

http://dx.doi.org/10.1016/j.jns.2017.02.047
0022-510X/

[37,48,61]. Thus, the agents that affect cAMP levels (e.g. phosphodiesterase, the main enzyme that degrades cAMP and reduces its concentration) [34], may influence LFS' anticonvulsant action. Adenosine receptors also exert their main effects through changes in cAMP levels. Adenosine A_1 receptors mediate inhibitory neuromodulation by coupling to inhibitory $G_{i/o}$-containing G proteins (for review, see: [7,16,32]). Therefore, regulators of G-protein signaling (RGS) proteins, which regulate the activity of G proteins, may be involved in the anticonvulsant actions mediated by adenosine A_1 receptors (for review, see: [14,36,50](.

RGS proteins modulate the G-proteins function by activating the intrinsic GTPase activity of α subunits, thereby reduce the duration of the activated GTP-bound state of the α subunit and inhibit G-protein function (for review, see: [14,36,50](. There are >30 members in the mammalian RGS family [67], and at least 10 different kinds of RGS proteins are expressed in the brain [62]. RGS2, which is expressed at high level in the hippocampus, increases the GTPase activity of $G_{\alpha s}$ protein. In addition, RGS2 can inhibit adenylyl cyclase and reduce the cytoplasmic concentration of cAMP [31]. RGS4 and RGS10 are also expressed at high levels in the hippocampus [51] and dentate gyrus [23] respectively. However, they increase GTPase activity of α subunit of $G_{i/o}$ proteins and thus reduce the inhibitory effect of $G_{i/o}$ on adenylyl cyclase and attenuate the decreasing effect of $G_{i/o}$ on cAMP concentration [23,51].

RGS protein expression is influenced by seizures. It has been shown that the gene expression of RGS10 decreases, but of RGS4 increases in the hippocampus following seizure induction [24,41]. These proteins are also involved in synaptic plasticity [31], presynaptic inhibition and thereby the regulation of neurotransmitter release [10]. Therefore, any changes in the expression of RGS proteins may have a role in the anticonvulsant action of LFS. In an attempt to make a better definition of cAMP's role as a main molecule involved in G_s and/or G_i protein-dependent signaling pathway in mediating the antiepileptogenic effect of LFS, first we investigated the effect of rolipram, a phosphodiesterase 4 inhibitor (that leads to an increase in cAMP concentration) on behavioral seizure severity and field potential recording changes following LFS antiepileptogenic action during a semi-rapid perforant path kindling procedure. Then we studied the possible changes in the expression of G_s and $G_{i/o}$ α subunits, RGS2, RGS4 and RGS10 proteins following LFS administration during kindling.

2. Material and methods

2.1. Animals

Adult male Wistar rats (8–9 weeks old), obtained from Pasteur institute of Tehran, Iran, were maintained in a colony room kept at a constant temperature on 12 h light/12 h dark schedule. The light phase was started at 7.00 a.m. Animals were individually housed in plastic cages with woodchip bedding and permitted free access to food and water. Experiments were done during the same time period (8:00 a.m. to 2:00 p.m.) to avoid the bias of circadian rhythms. This study was carried out in strict accordance with the ethical guidelines set by the "Ethical Committee of Faculty of Medical Sciences, Tarbiat Modares University" that are completely in accord with the "NIH Guide for the Care and Use of Laboratory Animals". All surgeries were performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering and the number of used animals.

2.2. Surgical procedures

Surgical procedure was done as explained previously [45]. Under sodium pentobarbital (50 mg/kg, i.p.) anesthesia, animals underwent stereotaxic implantation of a bipolar stimulating electrode in the perforant path (coordinates: A, −6.9 mm; L, 4.1 mm; and, V, 2–2.5 mm below dura) and a monopolar recording electrode in the dentate gyrus (coordinates: A, −2.8 mm; L, 1.8 mm; and, V, 2.5–3 mm below dura) of the right hemisphere [52]. Electrodes (stainless steel, teflon coated, 127 μm in diameter, A.M. Systems Inc., USA) were insulated except at their tips. The depth of the recording and stimulating electrodes was adjusted to maximize the population spike (PS) amplitude in the dentate gyrus in response to the perforant-path stimulation. Selective stimulation of the perforant path afferent fibers was confirmed by observing the paired pulse depression in response to paired pulses separated by 30–50 ms and recording the paired pulse facilitation in response to paired pulse stimulation with interpulse interval of 70 ms.

A 23-gauge guide cannula was also implanted in the right lateral ventricle (coordinates: A, −0.8 mm; L: ±1.4 mm and 2.6 mm below dura). Stainless steel screws were positioned in the skull above the frontal and occipital cortices and served as reference and ground electrodes. All electrodes were connected to pins of a lightweight multichannel miniature socket as a head-stage and fixed on the skull with dental acrylic. The experiments were started after at least 10 days of post-surgical recovery.

2.3. Stimulation and recording

The electrophysiological recordings were performed after the animal was transferred from the home cage to the recording box (30 × 30 × 30 cm) placed in a Faraday's cage. The rat was allowed to move freely in the recording box when its head-stage was connected to a flexible, shielded cable. Evoked responses were collected while the rat was motionless and awake with its eyes open. Input/output test was performed to determine the test pulse intensity used in the subsequent experiments. For input/output test, single 0.1 ms monophasic square wave pulses were delivered to the perforant path through a Nihon Kohden stimulator (Japan, Tokyo) and a Nihon Kohden SS-202J constant-current stimulus isolation unit (Japan, Tokyo) every 10 s at different intensities (100 μA–800 μA) while the evoked field potentials were monitored in the dentate gyrus.

For each time-point, twelve evoked responses were averaged. Both population- excitatory post-synaptic potential (pEPSP) slope and PS amplitude were monitored. The slope of pEPSP was determined at a fixed interval beginning approximately 0.25 ms after response onset and continuing within approximately 0.20 ms before PS onset. The PS amplitude was calculated by averaging the height from the peak of the pEPSP to maximum downward deflection of the PS (i.e. (b + c) / 2 in right upper trace in Fig. 3A). By means of input/output test the maximum PS amplitude was determined for each individual animal. All potentials employed as baseline criteria were evoked at a stimulus intensity which produced 50% of this maximum response (i.e., test pulse). The measured test pulse for different animals was between 100 and 500 μA. The evoked responses were continuously monitored, amplified and digitized (at 10 kHz) using a PC-based data acquisition system (D3107; ScienceBeam Co., Tehran, Iran). Then, they were averaged and finally stored on hard disk using a custom designed software (ScienceBeam Co., Tehran, Iran).

2.4. Semi-rapid kindling procedures

The semi-rapid kindling procedure was done as previously explained [45,55]. After ten days post-surgical recovery, the afterdischarge (AD) threshold was determined by 1 ms monophasic square wave of 50 Hz with 3 s train duration. The stimuli were initially delivered at 30 μA and then the stimulus intensity was increased in 10 μA steps at 5 min intervals. The minimum intensity sufficient to induce AD for at least 8 s was designated as the AD threshold, and used for stimulation. The AD threshold intensity ranged from 50 to 150 μA. Rats were stimulated at the AD threshold twelve times per day at 5 min intervals. The summation of the AD duration (ADD) after 12 daily stimulations was measured and reported in a cumulative manner. The behavioral progression of kindling (stages 1–5; according to Racine scores [54]) was also monitored.