Neuroscience 158 (2009) 1632–1643

THE ROLE OF ADENOSINE A_1 RECEPTORS IN MEDIATING THE INHIBITORY EFFECTS OF LOW FREQUENCY STIMULATION OF PERFORANT PATH ON KINDLING ACQUISITION IN RATS

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Abstract—Low frequency stimulation (LFS) has an inhibitory effect on rapid perforant path kindling acquisition. In the present study the role of adenosine A_1 and A_{2A} receptors in mediating this inhibitory effect was investigated. Rats were kindled by perforant path stimulation using rapid kindling procedures (12 stimulations per day). LFS (0.1 ms pulse duration at 1 Hz, 200 pulses, and 50–150 μA) was applied to the perforant path immediately after termination of each rapid kindling stimulation. 1,3-Dimethyl-8-cyclopenthylxanthine (CPT; 50 μM), a selective A_1 antagonist and ZM241385 (ZM, 200 μM), a selective A_{2A} antagonist were daily microinjected into the lateral ventricle 5 min before kindling stimulations. LFS had an inhibitory effect on kindling development. Pretreatment of animals with CPT reduced the inhibitory effect of LFS on kindling rate and suppressed the effects of LFS on potentiation of population EPSP during kindling acquisition. In addition, CPT was able to antagonize the effects of LFS on kindling-induced increase in early (10–50 ms intervals) and late (300–1000 ms intervals) paired pulse depression. ZM pretreatment had no effect on antiepileptogenic effects of LFS in kindling acquisition. In addition, LFS prevented the kindling-induced elevation of cyclic AMP (cAMP) levels in kindled animals. Based on these results, we suggest that the antiepileptogenic effects of LFS on perforant path kindling might be mediated through activation of adenosine A_1, but not A_{2A} receptors. Moreover, modulation of cAMP levels by LFS may potentially be an important mechanism which explains the anticonvulsant effects of LFS in kindled seizures.

Key words: seizure, brain stimulation, A_1 receptor, A_{2A} receptor, dentate gyrus.

Electrical stimulation for the treatment of pharmaco-resistant epilepsies is now being widely studied both clinically and experimentally (Velasco et al., 2001; Richardson et al., 2003; Goodman et al., 2005). There are a large number of studies showing the antiepileptic effects of low-frequency stimulation (LFS) applied in kindled animals and epileptic humans. Previous studies have demonstrated that in adult and immature animals (Velisek et al., 2002; Yamamoto et al., 2002; Goodman et al., 2005), as well as in patients with mesial temporal lobe epilepsy (Yamamoto et al., 2002), LFS (0.9–3 Hz) could produce inhibitory effects in epileptic activity (Weiss et al., 1995; Velisek et al., 2002; Yamamoto et al., 2002). On the other hand, it has also been shown that high-frequency stimulation exerts a potential efficacy in treatment of patients with epileptic seizures (Boon et al., 2007a,b; Van Roost et al., 2007). However, because of its less neural injury and a smaller amount of energy consumption, LFS has been suggested to be a better alternative therapy for epileptic disorders (Durand and Bikson, 2001).

LFS application depotentiates the synaptic potentiation in the amygdala efferent transmission induced by partial kindling and avoids the changes resulting from the kindling phenomenon in cats and rats (Adamec, 1999; Adamec and Young, 2000). Our previous study also showed that application of LFS during perforant path kindling retarded the kindled seizures development, inhibited the kindling-induced potentiation in perforant path–dentate gyrus synapses and prevented the increase in paired-pulse depression (Mohammad-Zadeh et al., 2007).

The precise antiepileptogenic mechanism of LFS is unknown. Application of LFS depotentiates the basal synaptic transmission after long-term potentiation (LTP) induction (Kulla et al., 1999; Mohammad-Zadeh et al., 2007). Since the kindling shares several features with LTP (Cain, 1989; Mohammad-Zadeh et al., 2007), similar mechanisms may account for the suppressing effect of LFS on LTP (depotentiation) and kindled seizures. In spite of many reports on the mechanism of LFS induced-depotentiation and/or long-term depression (LTD), there are few if any studies showing the mechanisms by which the LFS induces antiepileptic effects during kindling acquisition.

In hippocampal slices, LFS application changes the release of some neurotransmitters and neuromodulators including adenosine (Manzoni et al., 1994; Fujii et al., 2000b). In addition, it has been shown that adenosine derivatives are frequency dependently released as co-transmitters at hippocampal synapses (Schubert et al., 1976; Wieraszko et al., 1989; Cunha, 2001). Kindling and LFS-induced anticonvulsant effects are also frequency dependent phenomena (Gaito, 1980; Gaito et al., 1980; Sato et al., 1990). Thus, endogenous adenosine may contribute to LFS-induced antiepileptic effects in kindled seizures.

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Abbreviations: ACSF, artificial cerebrospinal fluid; cADD, cumulative afterdischarge duration; cAMP, cyclic AMP; CPT, 1,3-dimethyl-8-cyclopenthylxanthine; KLFS, kindled+low frequency stimulation; LFS, low frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; pEPSP, population excitatory post-synaptic potential; PS, population spike; ZM, ZM241385.

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doi:10.1016/j.neuroscience.2008.11.008

Adenosine is well known to play an important role in the modulation of central synaptic transmission and neuronal excitability (Ribeiro, 1995; de Mendonca and Ribeiro, 1997). Adenosine receptors are classified into A_1 and A_2 (including A_{2A} and A_{2B}) receptors (Daly et al., 1983; Schulte and Fredholm, 2003; van Calker et al., 1978, 1979) which have inhibitory and stimulatory effects on the levels of cyclic AMP (cAMP), respectively (Londos et al., 1980). There is a high concentration of A_1 adenosine receptors in the hippocampus (Fastbom et al., 1987). A_{2A} receptors have also been shown to be present in this region (Sebastiao and Ribeiro, 1992; Cunha et al., 1994).

Adenosine and its derivatives modulate several forms of synaptic plasticity including LTP and depotentiation (de Mendonca and Ribeiro, 1997; Fujii et al., 1999). Regarding the similarities between LTP and kindling-induced potentiation (Cain, 1989), it seems logical to assume a role for adenosine in anticonvulsant effects of LFS. However, the role of adenosine receptors in the depotentiation of LTP is not completely understood. Some investigators have demonstrated a facilitatory role (Fujii et al., 1997), and others showed an inhibitory role for adenosine A1 receptors in depotentiation (de Mendonca et al., 1997). There are also controversial reports on the role of A_2 adenosine receptors in the LFS-induced depotentiation (Fujii et al., 2000b; Huang et al., 1999).

In addition, there are many reports showing that A_1 receptor activation has antiepileptic effects in kindled seizures (Dragunow, 1988; Fredholm, 2003; Gouder et al., 2003; Mohammad-Zadeh et al., 2005). However, controversial results exist about the role of adenosine A_{2A} receptors in these seizures (Adami et al., 1995; Huber et al., 2002; Zeraati et al., 2006; Hosseinmardi et al., 2007). Changes in the concentration of cAMP, which occur following adenosine receptor activation, can also affect the seizure severity. The analogues of the nucleotide, db-cAMP, have been shown to be epileptogenic following intracerebral injection in rats (Kuriyama and Kakita, 1980; Itagaki, 1983).

Therefore, in the present study, we investigated the role of A_1 and A_{2A} receptors in mediating the anticonvulsant effect of LFS on perforant path-kindling acquisition by using selective antagonists of these receptors. Meanwhile, the changes in cAMP, as the main target second messenger of adenosine receptors, were also investigated following LFS application.

EXPERIMENTAL PROCEDURES

Animals

Eighty eight 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/dark schedule. The light phase was started from 7:00 a.m. Animals were individually housed in plastic cages with woodchip bedding and permitted free access to food and water. Efforts were made to minimize the animal suffering and the number of animals used. All studies were performed in accordance with the ethical guidelines set by the "Ethical Committee of School of Medical Sciences, Tarbiat Modares University" that were completely coinciding with the "NIH Guide for the Care and Use of Laboratory Animals." All experiments were done at the same time (8:00 a.m. to 2:00 p.m.) to avoid the bias of circadian rhythms.

Surgical procedures

Under sodium pentobarbital anesthesia (50 mg/kg, i.p.) animals underwent stereotaxic implantation with 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 (according to the atlas of Paxinos and Watson (1985)). Electrodes (stainless steel, Teflon coated, 127 μm in diameter, A.M. Systems, Inc., Carlsborg, WA, 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 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). The incisor bar was set 3.3 mm below interaural line. 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. Electrophysiological experiments were done after at least 10 days of recovery.

Stimulation and recording

All the recordings were performed after the rat had been transferred from the home cage to a recording box (30×30×30 cm) in the Faraday cage. The head-stage of the rat was connected to a flexible, shielded cable. The rat was allowed to move freely in the recording box. Evoked responses were collected while the rat was motionless and awake with its eyes open.

Input/output curves

Input/output curves were generated to establish the test intensity used in the subsequent experiments. For input/output tests, single 0.1 ms monophasic square wave pulses were delivered through Nihon Kohden stimulator (Japan, Tokyo) and Nihon Kohden SS-202J constant-current stimulus isolation unit every 10 s and applied at varying intensities (100 μA–800 μA) to the perforant path while the evoked field potentials were monitored in the dentate gyrus. For each time-point, 12 evoked responses were averaged. Both population excitatory post-synaptic potential (pEPSP) slope and PS amplitude were monitored. The PS amplitude was measured by averaging the height from the peak of the pEPSP to the base of PS, as shown in Fig. 4A. By means of input/output curve the maximum PS amplitude was determined for each animal and 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. Responses were evoked, amplified, digitized (at 10 kHz) using a PC-based data acquisition system (D3107, World of Science Instruments Co., Tehran, Iran) and custom-designed software, averaged and were continuously monitored and stored on disk.

Rapid kindling procedures

Rapid kindling procedure was done as explained previously (Mohammad-Zadeh et al., 2007; Sadegh et al., 2007). Following 10 days' postsurgical recovery, afterdischarge threshold was deter-