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Comparison between standard protocol and a novel window protocol for induction of pentylenetetrazol kindled seizures in the rat

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Summary Experimental models of epilepsy, including pentylenetetrazol (PTZ) chemical kindling, are very important in studying the pathophysiology of epilepsy. The aim of the present study was to provide behavioral, electrophysiological and molecular evidences to confirm the similarities between standard and a modified protocol named window- (win-) PTZ kindling method. Standard PTZ kindling model was induced by injection of PTZ (37.5 mg/kg) every other days. In win-PTZ kindling method, animals received 4 initial PTZ injections and the time of 3 last PTZ injections were determined according to the number of PTZ injections in standard PTZ kindling model. The behavioral signs of kindled seizures were observed for 20 min after each PTZ injection. Field potential recordings were done from the dentate gyrus granular cells following perforant path stimulation. In addition, the expression of γ_2 subunit of $GABA_A$ receptor, NR_2A subunit of NMDA receptor, adenosine A_1 receptor, α-CaMKII and GAP-43 were evaluated in the hippocampus and dentate gyrus using RT-PCR technique. All the animals in win-PTZ kindling method group achieved fully kindled state after 3 last PTZ injections. There was no significant difference in population spike amplitude and expression of the mentioned genes during kindling acquisition between standard PTZ kindling model and win-PTZ kindling method. The similarities in electrophysiological and molecular parameters remained after 8 days post fully kindled state. Obtained data showed the similarities between this win-PTZ kindling method and standard PTZ kindling model. Thus, it may be suggested that not all PTZ injections are need for induction of PTZ induced fully kindled state.

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Introduction

Animal models of epilepsy are widely used to investigate the epileptogenic mechanisms or to test the efficacy of new antiepileptic compounds. Temporal lobe epilepsy is among the most common epileptic disorders (McNamara, 1999) and one of the most common used models of temporal lobe epileptic seizures is chemical kindling. In chemical kindling the gradual development of electrographic and behavioral seizures occurs with repeated stimulation of animal by convulsive chemicals such as pentylenetetrazol (PTZ). The main features of chemical kindling are the progressive development of behavioral seizures, a reduction in seizure threshold, and a maintained heightened sensitivity to the seizure-inducing stimulus (Gilbert and Goodman, 2006). These features are accompanied with electrophysiological and molecular changes in different brain areas including hippocampus and dentate gyrus.

Dentate gyrus is among the most sensitive brain regions to kindling stimulations (Morimoto et al., 2004; Ang et al., 2006). PTZ kindling increases the amplitude of population spikes (PS) in the dentate gyrus granular cells (Adamec et al., 1981; de Jonge and Racine, 1987; Maru and Goddard, 1987; Gilbert, 1991; Robinson et al., 1991; Fathollahi et al., 1997; Ruthrich et al., 2001).

In addition to electrophysiological changes, several changes occur in gene expression of the proteins involved in PTZ kindling acquisition and/or maintenance, including: (a) γ_2 subunit of $GABA_A$ receptor, the most abundant subunit of this receptor in the dentate gyrus (Nishimura et al., 2005), (b) NR_2A subunit of NMDA receptors, a regulatory subunit that determine distinct functional properties of NMDA receptors (Ekonomou and Angelatou, 1999; Zhu et al., 2004; Yashiro and Philpot, 2008), (c) adenosine A_1 receptor, the main receptor for mediating the anticonvulsant action of adenosine (Fredholm and Lindstrom, 1999; Tchekalarova et al., 2005), (d) Ca^{2+}-calmodulin kinase II (CaMKII), a key transducer of extracellular signals that plays a role in the achievement of PTZ kindling (Ates et al., 2005) and (e) growth associated protein-43 (GAP-43), a marker for neuronal plasticity that may play a role in seizure induction (Spencer et al., 1992; Oh et al., 2002).

Standard PTZ chemical kindling is elicited by repeated intraperitoneal (i.p.) injections of a subconvulsant dose of PTZ once every 48 h until the animal being fully kindled. In this model, PTZ is chronically injected during a long time to induce a fully kindled state in animals. Recently, Schmoll et al. (2003) have suggested that there is a critical time window after the first PTZ injection during which a cycle of gene expression will be completed and at the end of this time window, application of a subconvulsive dose of PTZ can evoke seizure activity. They showed that application of PTZ initiates a chain of events with a 25-days-period, at the end of which the rat brain is highly susceptible to the development of generalized seizures induced by a subconvulsive dose of PTZ.

Furthermore, our preliminary experiments showed that following the 4 initial injections of PTZ a series of changes occur which make the animals more susceptible to seizure induction so that after elapsing a time window, application of 3 subthreshold doses of PTZ would lead to fully kindled seizures (i.e. three repetitive stage 4 or 5 seizures). We named this method ''window- (win-) PTZ kindling method''. The aim of the present study was to provide evidences for confirming the behavioral, electrophysiological and molecular similarities between the ''win-PTZ kindling method'' and ''standard PTZ kindling model''.

Materials and methods

Animals

107 adult male Wistar rats (8–9 weeks old) obtained from Pasteur institute of Iran were maintained in a colony room kept at $23 \pm 2\,^{\circ}C$ temperature on 12:12 light:dark schedule with light from 7:00 am to 7:00 pm. Housing conditions were the same for all animals throughout the study. Single animals were housed in standard 30 cm × 45 cm × 20 cm cages and wooden chips were used as bedding in the cages. The rats were supplied with water and standard pelleted diet (Pasture institute, Iran) ad libitum. All studies were performed in accordance with the ethical guidelines set by the ''Ethical Committee of Faculty 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 in the morning to avoid the bias of circadian rhythms.

Behavioral experiments

Animals were kindled by i.p. injection of PTZ (37.5 mg/kg; 0.1 ml/100 g body weight) every second day. Following each PTZ injection, the behaviors of animal were observed for a period of 20 min. The seizure stages were classified as follows: 0: no response; 1: ear and facial twitching; 2: convulsive waves through the body; 3: myoclonic jerks, rearing; 4: clonic-tonic convulsions, turnover into side position; 5: generalized clonic-tonic seizures, loss of postural control (Corda et al., 1990). Animals were considered fully kindled after manifestation of three consecutive stage 4 and/or 5 seizures.

Electrophysiological experiments

For electrophysiological recordings, animals underwent stereotaxical surgery under sodium pentobarbital anesthesia (50 mg/kg, i.p.) as explained previously (Jahanshahi et al., 2009). Briefly, the animals were implanted with a monopolar recording electrode in the dentate gyrus (coordinates: A, −2.8 mm; L, 1.8 mm; and, V, 2–2.7 mm below dura) and a bipolar stimulating electrode in the perforant path (coordinates: A, −6.9 mm; L, 4.1 mm; and, V, 1.7–2.5 mm below dura) of the right hemisphere (according to the atlas of Paxinos and Watson (1986)). The vertical coordinates of the electrodes were adjusted so that maximum PS amplitude in the dentate gyrus was recorded in response to the perforant path stimulation.

Freely moving electrophysiological recordings were performed after ten days post-surgical recovery period. To determine the test pulse intensity, input/output curves were obtained by application of single monophasic square wave pulses (0.1 ms pulse duration, at the frequency of 0.1 Hz

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