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Neurochemical evidence based suggested therapy for safe management of epileptogenesis

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ABSTRACT

Most of the clinically available antiepileptic drugs have only antiseizure effects and are reported unable to prevent epileptogenesis. In the past decade, several drugs underwent clinical trials for management of epileptogenesis, but none of the drugs tested was found effective. One of the major lacunas is availability of appropriate preclinical approaches to delineate mechanisms of epileptogenesis. Thus, the present study attempts to suggest a neurochemistry based approach for safe management of epileptogenesis. The altered neurochemical milieu in amygdala, cortex and hippocampus areas of the mice brain in naïve, kindled and kindling resistant animals has been delineated. The endogenous natural antiepileptogenic neurochemical defense mechanism observed in kindling resistant animals may uncover neurochemical mechanisms of epileptogenesis and in turn suggest us novel interventions for safe management of epileptogenesis. The kindling epileptogenesis was carried out in two month old male Swiss albino mice by administering subconvulsive pentylenetetrazole (35 mg/kg; i.p.) at an interval of 48 ± 2 h for 42 days. 2 h after the last pentylenetetrazole injection, the animals were subjected to behavioral evaluations. Four hours after behavioral evaluation, all animals were euthanized and discrete parts of brain (amygdala, cortex and hippocampus) were harvested for neurochemical analysis. Results revealed that 60% of animals responded to kindling as observed with decreased seizure threshold, while the rest were found resistant. The kindled animals were found to be associated with anxiety, depression and cognitive impairment; while in kindling resistant animals no such behavioral deficits were observed. The neurochemical analysis revealed that in kindled animals altered glutamate–GABA neurotransmission, and decreased taurine, glycine, D-serine, monoamine levels with elevated indoleamine 2,3-dioxygenase activity were observed, which may be convicted for progression of kindling epileptogenesis. However, in kindling resistant animals elevated GABA, taurine, tryptophan, serotonin, glycine, and D-serine levels with decreased indoleamine 2,3-dioxygenase activity were observed as natural endogenous antiepileptogenic mechanisms, which may be foreseen as safe pharmacological targets for management of epileptogenesis.

1. Introduction

Epilepsy is one of the major neurological disorders characterized by spontaneous recurrent seizures (SRS) due to excessive neuronal discharges [1]. Although more than ten new antiseizure drugs (ASDs) have been developed in the past decade, epilepsy remains resistant to drug therapy in about one-third of patients. Approximately 20% of patients with primary generalized epilepsy and up to 60% of patients having focal epilepsy develop pharmacoresistance during their medication course [2]. The development of pharmacoresistance during medication course clearly indicates lack of antiepileptogenic effects of available ASDs.

There is no incontrovertible evidence supporting the idea that ASDs administered during the latent period following status epilepticus (SE) prevented the development of epilepsy [3]. However, some studies indicated that development of epilepsy may be delayed or the severity of SRS may be reduced by such treatment. The promising preclinical findings of levetiracetam [4,5] and topiramate [6,7] prompted the clinical trials in which these ASDs were examined for antiepileptogenic effects in patients with traumatic brain injury [8–11]. No other ASD has shown promising results to be tested in clinical trials [12]. To meet this clinically unmet need, various molecules other than ASDs such as anti-inflammatory, neuromodulators, neuroprotective agents underwent clinical trials for management of epileptogenesis but none could get into clinics [12,13]. One of the major lacunas in this regard is non-availability of appropriate preclinical approaches to delineate pathological mechanisms of epileptogenesis.

Numerous pathological alterations occur simultaneously during the epileptogenic cascade, so it is most certainly not be possible to halt

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epileptogenesis by targeting only one of these pathological mechanisms [13]. Thus understanding the basic mechanisms of epileptogenesis and epileptogenicity represents priority for epilepsy research [12]. In this respect, clinical studies have very limited capacity to delineate such basic mechanisms. However, animal models are invaluable tools of investigation to uncover these mechanisms [14]. *Proechimys* (neotropical rodents), a rodent of genus of South American spiny rats of the family Echimyidae have been investigated in different experimental epilepsy models and have been proposed as an animal model resistance to epileptogenesis. The explorations of different molecular pathways in these animals have suggested various natural endogenous antiepileptogenic mechanisms [14].

In view of this approach, we warrant to explore pertinent neurochemical alterations in pentylenetetrazole kindling epileptogenesis resistant animals. As reported in most of our previous studies [15–17] as well as by many other laboratories [18–24] 20–40% of animals were resistant to developing pentylenetetrazole kindling epileptogenesis. Chemical or electrical kindling in mice or rat represents features of focal epilepsy with secondary generalization in humans, involving amygdala, hippocampus, and cortex as major brain parts involved in epileptogenesis [22,25]. It is a widely exploited experimental model of epilepsy defined by a progressive increase in electrographic and behavioral seizures [26,27]. The repeated application of an initially subconvulsive chemical or electrical stimulation gradually lowers the seizure threshold which leads to development of seizure [26–29]. The continued repeated administration of subconvulsant pentylenetetrazole dose then results in enhancement of seizurogenic responses associated with abnormal neurogenesis [30–32]. Pentylenetetrazole-induced kindling epileptogenesis is a long-lasting phenomenon, remaining for at least ten months after drug discontinuation [33–35]. Furthermore, kindling epileptogenesis has been suggested more predictive for searching novel therapies for anti-epileptogenesis than post-SE models [12]. Thus, the neurochemical exploration in pentylenetetrazole kindling epileptogenesis resistant animals in comparison to naïve and kindled animals may represent a relevant tool to investigate the endogenous defense mechanisms for safe and effective management of epileptogenesis [14].

Neurotransmitters such as monoamines (norepinephrine, serotonin, dopamine, kynurenine) and amino acids (glutamate, GABA, tryptophan, taurine) have been reported to regulate functional activity of neurons and modulate synaptic transmission [36]. Altered neurochemical milieu after the brain/epileptic insult may disrupt adult hippocampal neurogenesis, which could alter the organization and function of brain circuitry, eventually leading to manifestation of neurological disorders including epilepsy [37]. Targeting altered neurochemical milieu may have normalizing effect on aberrant neurogenesis, highlighting its potential significance for safer antiepileptogenic effects [36–38]. At present, most therapeutic approaches for the treatment of neurological disorders such as antidepressants or antiparkinsonian drugs target only to restore the altered neurochemical environment, underpinning the pathophysiological role of altered neurochemical milieu in progression of disease [15]. Therefore, we hypothesized that comparison of neurochemical milieu or environment between naïve, kindled, and kindling epileptogenesis resistant animals in amygdala, cortex and hippocampus may be useful to reveal some safe and effective neurochemical target/s against epileptogenesis.

2. Material and methods

2.1. Animals

This study was carried out in male Swiss albino mice weighing 22–28 g, obtained from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana. The animals were housed in groups of 6 mice/cage at an ambient room temperature (22 ± 2 °C), with a relative humidity of 50 ± 5% under 12:12 h dark light cycle (lights on at 07:00 h). Food and water were available ad libitum except during specific experimental protocols. The experimental protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) and the care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India vide protocol approval no. 107/99/CPCSEA/-2013-27.

2.2. Drug and chemicals

All chemicals employed for the biochemical and neurochemical estimations were of analytical grade and were freshly prepared before each experiment. Pentylenetetrazole, kynurenine, dopamine, serotonin were procured from Sigma-Aldrich, Co., St. Louis, MO, USA. Taurine, D-serine, and glycine were procured from HiMedia Laboratories, Mumbai, India. Tryptophan was procured from Loba Chemie, Mumbai, India. Gamma-aminobutyric acid (GABA) was acquired from Central Drug House, New Delhi, India; glutamate from S.D. Fine Chemicals, Mumbai, India; high performance liquid chromatography (HPLC)-grade methanol and heptane sulfonic acid were procured from Merck Specialties, Mumbai, India. Norepinephrine was obtained as a gift sample from Troikaa Pharmaceuticals Ltd., Dehradun, India. Perchloric acid was purchased from Spectrochem, Mumbai, India.

2.3. Pentylenetetrazole kindling epileptogenesis

Pentylenetetrazole induced kindling epileptogenesis was induced in male Swiss albino mice employing the method previously validated in our laboratory [15–17]. Pentylenetetrazole dissolved in normal saline was injected intraperitoneally (i.p.) at a sub-convulsive dose of 35 mg/kg at 48 ± 2 h intervals. After each injection, the mice were placed individually into Plexiglas cages (20 × 20 × 30 cm) and observed for 30 min. The intensity of the convulsions was registered according to modified Racine's scale [15]; stage 0: no response; stage 1: hyperactivity, restlessness and vibrissae twitching; stage 2: head nodding, head clonus and myoclonic jerks; stage 3: continuous myoclonic jerk with tail rigidity; stage 4: generalized limbic seizures with kangaroo posture or violent convulsions; stage 5: generalized tonic–clonic seizures with falling and stage 6: hind limb extensor. The transition of the convulsion intensity from the 4th to 5th stage reflected the generalization of the convulsive activity, manifested as tonic–clonic convulsions. The animals were considered kindled after appearance of stage 5 seizures on five consecutive subconvulsive pentylenetetrazole administrations during kindling epileptogenesis.

2.4. Experimental protocol

A total of 30 animals were employed in this study. The group I: NAIVE, consisted of naive animals (non-kindled, n = 10) and the remaining 20 animals were subjected to kindling. Group II constituted successfully kindled animals (n = 12). Group III constituted kindling epileptogenesis resistant animals (n = 8). Two hours after the last pentylenetetrazole subconvulsive dose on day 42 (after 21st injection), once their locomotor activity became normalized the animals were subjected to behavioral evaluations for anxiety, depression and cognition. 4 h after behavioral evaluations (for excluding any potential effect of behavioral evaluations on brain neurochemistry), all animals were euthanized and discrete parts of mice brain (amygdala, cortex, and hippocampus) were separated in a cold room (maintained at 4 °C) for neurochemical analysis. All these experiments were carried out in the same time of the day to exclude effect of circadian rhythm on altered biochemical and neurochemical milieu in different animal groups. The pictorial representation of protocol is shown in Fig. 1.