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Effects of topiramate on seizure susceptibility in kainate-kindled rats: Involvement of peripheral-type benzodiazepine receptors

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Summary This study was aimed to quantitatively evaluate the effects of topiramate (TPM) on seizure susceptibility and hippocampal peripheral-type benzodiazepine receptors (PBRs) in the kainic acid (KA) model of temporal lobe epilepsy. Male rats were randomized into saline control group, KA group, KA/TPM low dose group and KA/TPM high dose group. Three weeks after single injection of KA (10 mg kg^{-1}, sc), the effects of TPM were tested at two doses (10 and 30 mg kg^{-1}, sc) once a day for 1 week in KA/TPM low dose group or KA/TPM high dose group, respectively. Rats in KA group received comparable injections of saline. Four weeks after initial KA injection, a subconvulsant dose KA (5 mg kg^{-1}, sc) was administered in rats in these three groups. Rats in saline control group received equal volume of saline. All animals were decapitated and hippocampus synaptosomes were purified 180 min after behavioral observation. PBRs specific binding sites were assessed by an in vitro binding technique utilizing the highly selective ligand [^{3}H]PK11195. Seizure threshold was elevated and specific PBRs binding in hippocampus was decreased by TPM in dose-dependent manner. Specific PBRs binding in hippocampus was significantly related to seizure latency and seizure intensity. These results suggest that TPM can reduce the susceptibility to seizures in KA-kindled rats and its anticonvulsant effect seems resulting from, at least in part, the reduced PBRs binding after treatment. These results also support the hypothesis that PBRs represent a novel target for antiepileptic drug development.

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Introduction

The peripheral-type benzodiazepine receptors (PBRs) are distinct from the central-type benzodiazepine receptors structurally, functionally and pharmacologically. PBRs are a multimeric complex found mainly on the outer mitochondrial membrane of astrocytes. PBRs may also be colocalized with activated microglia in injured brain tissue. PBRs are composed with three subunits, an 18 kDa isoquinoline carboxamide-binding protein, a 34 kDa voltage-dependent anion channel and a 30 kDa adenine nucleotide carrier. PBRs have been identified with various physiological functions, such as steroidogenesis, respiration, cell growth and differentiation. Increased PBRs expression was associated with both microglial activation and astrogliosis enhancement. Increased binding sites for PBRs ligands have been described in a wide range of neurological disorders including seizure in both human and animal.[1]

In spite of the rapid development of new antiepileptic drugs over the past 20 years, approximately 30% of patients are still refractory to all types of treatments. Thus, the searches for new mechanisms of action that can regulate cellular excitability are vitally needed. Topiramate (TPM) is a widely used antiepileptic agent. In vitro studies suggested that TPM affects neuronal activity and produces its antiepileptic effects by several mechanisms, but which precise mechanism of function is poorly understood. The aim of this study was to measure PBRs expression in hippocampus from KA-induced epileptic rats and test the effects of TPM on seizure threshold and PBRs.

Materials and methods

Materials

TPM was purchased from Janssen Pharmaceutical Ltd. [^{3}H]PK11195 (specific activity, 83.5 Ci/mmol; radiochemical purity, 97%) was purchased from PerkinElmer Life Sciences (Boston, MA). Kainic acid (KA), sucrose, bovine serum albumin, POPOP and unlabelled PK11195 were purchased from Sigma Chemical Co. (St. Louis, MO). PPO and Coomassie brilliant blue G-250 were purchased from Fluka Ltd. (Buchs, Switzerland). Triton X-100 was purchased from Amersham Pharmacia Biotech, Inc. (Uppsala, Sweden). Other chemicals, of reagent grade or better, were from standard commercial suppliers.

Preparation of the reagents

Sucrose buffer (0.25 M sucrose, 1 mM EDTA, 10 mM HEPES—NaOH, pH 7.4) and 0.8 M sucrose solution (0.8 M sucrose, 1 mM EDTA, 10 mM Tris—HCl, pH 7.4) were prepared with distilled water. PK11195 was dissolved in DMSO, and then diluted with phosphate-buffered saline (PBS) to produce test solution. PPO and POPOP dissolved in toluene and Triton X-100 were used as scintillates. TPM and KA were dissolved in saline at suitable concentrations for injection, respectively. All experiments were done under approved animal protocols.

Preparation of the rat model

Male Sprague—Dawley (SD) rats (180—220 g, $n = 24$) were provided by Beijing Experimental Animal Center (Beijing, China). The rats were housed solitarily in plastic cage under standard conditions (light/dark cycle, 7:00 a.m.—7:00 p.m. lights on), with food and water available ad libitum. Temperature (22 °C) and humidity (60%) were kept constant. All animals were acclimated to their home cages for at least 5 days before testing. Experiments were conducted between 9:00 a.m. and 3:00 p.m. in an experimental room. Animals were randomly divided into saline control group, KA group, KA/TPM low dose group and KA/TPM high dose group. All KA-treated rats ($n = 18$) received KA (10 mg kg^{-1}, 1 ml kg^{-1}, sc). The rats were immediately placed in individual Plexiglas containers (14 cm × 25 cm × 36 cm high) for behavioral observation. Three weeks after KA injection (10 mg kg^{-1}), the effects of TPM were tested at two doses (10 mg or 30 mg kg^{-1}, 1 ml kg^{-1}, sc) once a day for 1 week in KA/TPM low dose group and KA/TPM high dose group, respectively. Rats in KA group received comparable injections of saline. Four weeks after initial KA injection, KA (5 mg kg^{-1}, 1 ml kg^{-1}, sc) was injected in all KA-induced seizure rats. Rats in saline control group received equal volume of saline.

Behavioral observation

Occurrence of spontaneous recurrent seizures was video-recorded for 10 h following the initial KA treatment and for 180 min following the second KA treatment in all rats. Seizure threshold was based on the severity of the seizures and the latency from KA injection to seizures. The seizures were divided into five stages according to their severity as described previously.[2] Latency was measured as the time to onset of the first physical sign of seizures, forelimb clonus. Videotapes were reviewed and detected seizures were scored.

Preparation of synaptosomal fractions

Rats were anesthetized with pentobarbital sodium solution (50 mg/kg) and then decapitated after