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vGLUT2 heterozygous mice show more susceptibility to clonic seizures induced by pentylenetetrazol

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ABSTRACT

Glutamate, the most abundant excitatory neurotransmitter in the central nervous system, is well known to be implicated in epileptic seizures. Therefore, impairments in glutamate transport could have an involvement in the mechanism of epileptogenesis. The uptake of glutamate into synaptic vesicles is mediated by vesicular glutamate transporters (vGLUTs). There are three known vGLUT isoforms, vGLUT1–3. In this study, we are particularly interested in the vGLUT2 isoform. We investigated the possible role of vGLUT2 in pentylenetetrazol (PTZ)-induced seizure generation. Seizure threshold of PTZ was compared in vGLUT2 heterozygous knock out (HET) and wild type (WT) mice. In comparison with their WT littermates a lower dose of PTZ was needed in the vGLUT2 HET mice until the onset of the first myoclonic jerk. The threshold for PTZ-induced clonic seizure activity was also lower in the vGLUT2 HET mice. These results indicate, for the first time, that vGLUT2 is likely involved in the epileptogenesis of generalized seizures.

1. Introduction

Glutamate, the principal excitatory neurotransmitter in the central nervous system, undoubtedly plays a role in the initiation and spreading of seizure activity. Aberrant glutamate transport can seriously affect the processes of epilepsy (Danbolt, 2001). The excitatory amino acid transporters (EAATs) are important in clearing unbound glutamate from the extracellular space. Deletion of the glial high affinity glutamate transporters, GLT-1 (EAAT2) and GLAST (EAAT1), leads respectively to lethal seizures and high seizure susceptibility in mice. The expression profile of EAAC1 (EAAT3), one of the neuronal transporters, is reduced within hours following kainic acid-induced seizures (Sheldon and Robinson, 2007). Besides the EAAT family, there is also evidence that the vesicular glutamate transporter (vGLUT) family is implicated in epileptogenesis. These transporters load glutamate into vesicles for subsequent release during fast synaptic transmission (Takamori, 2006). Seal et al. (2008) recently reported that mice lacking vGLUT3 exhibit primary, generalized epilepsy.

In this study, we are particularly interested in the possible involvement of vGLUT2 in epilepsy. Immunoreactivity and expression profiles of vGLUT2 have already been examined in different models of epilepsy. In the Mongolian gerbil, vGLUT2 as well as vGLUT1 immunoreactivities were markedly enhanced in the molecular layer of dentate gyrus of seizure sensitive gerbils compared to seizure resistant gerbils. In line with these findings, treatment with valproic acid dramatically reduced vGLUT expressions in the seizure sensitive gerbils (Kang et al., 2005). In P20–30 methylazoxymethamol-exposed rats, vGLUT2 was strongly expressed in hippocampal heterotopic regions, whereas EAAC1 expression was decreased in these regions (Harrington et al., 2007). The Genetic Absence Epilepsy Rat from Strasbourg (GAERS) is recognized as a valid tool to investigate generalized absence seizures. The expression profile of vGLUT2 was increased in the cortex of adult GAERS, but not in the thalamus, while vGLUT1 expression appeared to be normal (Touret et al., 2007).

Pentylenetetrazol (PTZ) is a tetrazole derivative that has convulsant actions, presumably by impairing GABA-mediated inhibition through an action on the $GABA_A$ receptor. The behavioral and electroencephalogram (EEG) manifestations of PTZ-induced seizures in rodents suggest that the drug is a model of generalized seizures, both absence and generalized tonic–clonic seizures (Zhao and Holmes, 2006). In adult rodents, PTZ induces absence seizures at low dosage. In this type of seizure activity, the cortex, together with the reticular nucleus and the relay nuclei of the thalamus play

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a predominant role in the development of the spike and wave discharges (Van Luijtelaar and Sitnikova, 2006). Moderate doses of PTZ lead to forebrain-type clonic seizures, while high doses of PTZ induce brainstem-type tonic–clonic seizures and eventually generalized status epilepticus and death (André et al., 1998; Danober et al., 1997). When PTZ is given intravenously in rodents at relatively high concentrations, these animals show rapid onset of seizures, together with all typical successive phases of behavior (Keogh et al., 2005).

The upregulation of vGLUT2 immunoreactivities, as described above (Kang et al., 2005; Harrington et al., 2007; Touret et al., 2007), may all be causally or consequently related to the hyperexcitability and/or presence of seizures in these animal models.

In the present study, we investigated the seizure susceptibility of vGLUT2 heterozygous knock out (HET) mice, and their wild type (WT) littermates in the well-established PTZ model for generalized epilepsy. This will allow us to investigate whether a severe reduction in vGLUT2 protein in the HET mice can affect seizure generation and thus whether the vGLUT2 protein is crucially involved in the origin of generalized seizure activity.

2. Materials and methods

2.1. Animals

Five-month-old male vGLUT2 HET and WT littermates (30–37 g) derived from HET × HET breeding pairs were developed and provided by Moechars et al. (2006). Similar experiments could not be performed with homozygous vGLUT2 knock out mice, because complete loss of vGLUT2 is lethal.

All experiments were carried out according to the National Guidelines on Animal Experimentation and were approved by the Ethical Committee for Animal Experiments of the Vrije Universiteit Brussel. All animals were housed under standard laboratory conditions. Mice stayed in the animal house in standard breeding cage with food and water *ad libitum* and on a 10/14 h dark/light cycle (lights on between 07:00 and 21:00).

2.2. 24 h video electroencephalogram (EEG) telemetry monitoring

vGLUT2 HET and WT mice (both $n = 3$) were anesthetized by an intraperitoneal (i.p.) injection of a mixture of ketamine (Ceva, Brussel, Belgium)/Rompun (Bayer, St. Truiden, Belgium)/saline (Baxter, Lessines, Belgium). An incision was made in the abdomen and the scalp. A transmitter (model TA10EA-F20, Data Science International, St. Paul) was placed in the abdomen of the animals. After tunneling the electrical leads of the transmitter to the skull, the abdomen was sutured. Next, the animals were placed on a stereotaxic frame. Holes were made in the skull, in which screws, connected to the leads, were implanted. The screws were fixed in the skull at following coordinates: lateral, −2/+2; anterio-posterior, −2,1. The electrodes and screws were then covered with dental acrylic cement (Dentsply Caulk, Milford, USA), and the scalp was sutured. After the surgery, the mice were given ketoprofen (4 mg/kg, i.p.; Merial, Essex, United Kingdom) and were allowed to recover for 7 days in the animal house.

The basal EEG of the animals was measured every day during at least 3 h. Every 3 days, the basal EEG of the mice was also measured overnight for at least 12 h. Four weeks after surgery, these mice were subjected to PTZ-induced seizures (protocol described below). The obtained EEG spectra were analyzed using Notocord-hem Evolution® software (Notocord, Croissy, France).

2.3. Determination of convulsive parameters and seizure threshold

A separate experimental group of animals (vGLUT2 HET, $n = 8$; WT, $n = 8$) was used to determine seizure thresholds, based on the PTZ-induced stereotyped seizure behavior. These mice were not subjected to general anesthesia and did not undergo any surgical procedures.

The threshold for different phases of PTZ-induced seizure activity was determined by infusing PTZ through a 29G needle, attached to a polyethylene tubing (Smiths, Keene, USA), inserted into the tail vein of the animals. The needle was secured to the tail with a piece of tape. Afterwards, the animal was able to move freely in a cage made of Plexiglas. The PTZ (Sigma Chemical Company, St. Louis, MO, USA) solution (7.5 mg/ml) was infused into the tail vein at a constant rate of 150 μl/min, using a Hamilton syringe mounted to an infusion pump (CMA, Microdialysis, Solna, Sweden). The syringe was connected to the needle by polyethylene tubing.

The following endpoints were used to determine the seizure threshold: (1) myoclonic twitch; (2) onset of generalized clonic phase without loss of righting reflexes; (3) onset of generalized tonic phase; (4) death. Time was measured from the start of the PTZ infusion until the onset of these stages. The seizure thresholds were determined for each animal according to the following equation: dose (mg/kg) = duration of infusion (s) × rate of infusion (ml/min) × drug concentration (mg/ml) × 1000/(60 (s) × weight of mouse (g)).

2.4. Statistical analyses

The data were analyzed by using the non-parametric two-tailed Mann–Whitney test ($\alpha = 0.05$).

3. Results

3.1. EEG monitoring

Fig. 1A shows a representative example of the baseline EEG monitoring of a vGLUT2 HET mouse. The EEG pattern of the HET mice did not show any appearance of abnormalities, compared to the baseline EEG spectra of a WT littermate (data not shown).

After PTZ-infusion, both vGLUT2 HET and WT mice showed the same stereotyped behavior and comparable patterns of epileptiform activity were noted. The general pattern of activation included a first spike (myoclonic twitch), followed by regular spikes (clonic seizures), rhythmic spiking, spike and wave discharges and rapid sharp shaped spikes (tonic seizures) (Fig. 1B–D).

3.2. Determination of seizure threshold

After correlating the PTZ-induced stereotyped behavior response with the EEG spectra, the different behavior responses were scored in animals without EEG monitoring for seizure threshold determination. Continuous infusion of PTZ solution in the tail vein of the mice induced a first myoclonic twitch/jerk, sometimes accompanied with loss of reflexes, followed by forelimb clonus, tonic–clonic convulsions, tonic hind limb extension and death. In this study, only the clearest stereotyped seizures or endpoints were scored (Fig. 2).

As shown in Fig. 2A, the doses of PTZ needed to induce the first myoclonic twitch were significantly ($P = 0.003$) decreased in the vGLUT2 HET mice compared to their WT littermates. There was also a significantly ($P = 0.028$) decreased dose of PTZ necessary to induce forelimb clonus in the vGLUT2 HET animals compared to the WT mice (Fig. 2B). On the other hand, when the dose that induces tonic hind limb extension was compared between the two genotypes, no statistical difference ($P = 0.5$) was shown (Fig. 2C).

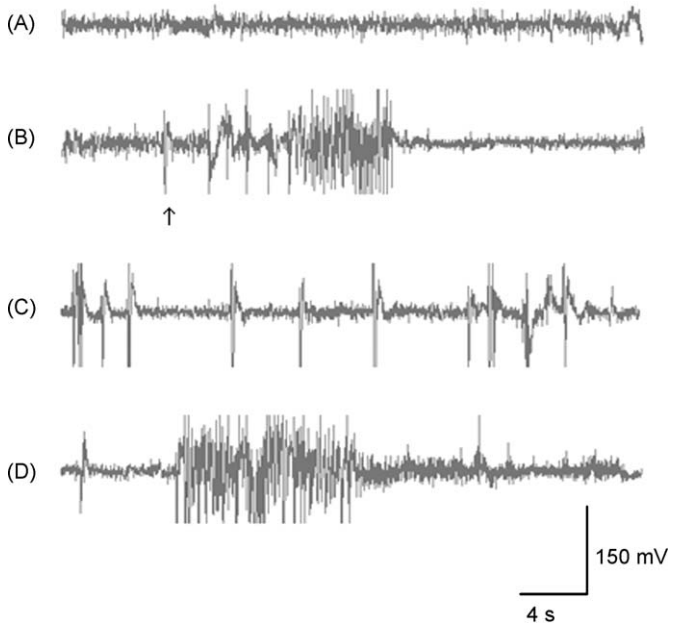

Fig. 1. Representative example of EEG recordings of the successive phases of seizure activity induced by i.v. infusion of PTZ. (A) Baseline EEG. (B) A first spike (myoclonic twitch (↑)), followed by multiple spikes (clonic seizures). (C) Regular spikes (clonic seizure activity). (D) Polyspikes (tonic seizures).