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Effects of conventional anticonvulsant drugs on generalized tonic-clonic seizures in Noda epileptic rats

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Summary Noda epileptic rats (NERs) present with clinico-pathological manifestations reminiscent of human generalized tonic-clonic epilepsy. Thus, this strain of rat has been a model of primary, generalized, tonic-clonic epilepsy. However, the infrequency of seizures in these rats makes the assessment of antiepileptic drugs (AEDs) difficult. Therefore, traditional AEDs have only been tested in NERs against audiogenic seizures evoked by weekly acoustic priming from 3 to 22 weeks of age or by using the kindling procedure in adult animals. Adult NERs are susceptible to changes in their environment, such as bedding replacement or unpleasant sensory stimuli. In the present study, traditional AEDs—phenobarbital (PB) and sodium valproate (VPA)—were evaluated against seizures evoked by strong environmental stimuli in mature NERs that had not been previously primed. The number of animals presenting with seizures decreased in a dose-dependent manner following administration of either PB (dose range 1.0—5.0 mg/kg) or VPA (50 and 100 mg/kg). Consequently, the utility of NERs as a model of generalized tonic-clonic epilepsy was confirmed. This type of protocol can be used to further evaluate AEDs and test effects of chronic administration of AEDs.

Abbreviations: AEDs, antiepileptic drugs; PB, phenobarbital; VPA, sodium valproate; NERs, Noda epileptic rats.

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Introduction

Pre-clinical evaluations of new antiepileptic drugs (AEDs) require experimental models to assess both their spectrum of antiepileptic activity and their safety (Leite and Cavalheiro, 1995; Löscher, 2002; White et al., 2005). The maximal electroshock (MES) and pentylenetetrazol (scPTZ) seizure models represent the two most widely used animal seizure models employed in the search for new AEDs (White et al., 2002). The MES test is thought to predict drugs preferentially active against generalized seizures of the tonic-clonic type, whereas the scPTZ test is used to identify those effective against generalized absence-type seizures (Leite and Cavalheiro, 1995). The 6-Hz psychomotor seizure model may represent a potential model for developing therapies for drug-resistant epilepsy (Barton et al., 2001; White et al., 2005). In contrast to these acute models, some genetic animal models have also been reported (Coenen et al., 1992; Inoue et al., 1994; Marescaux and Vergnes, 1995). Such models could also be used as chronic models in the search for novel therapies or to improve our understanding of the mechanisms of seizure generation (Coenen and Van Luijtelaar, 2003; Inoue et al., 1993). However, these models represent absence-type seizures.

Noda epileptic rats (NERs) were developed in the 1990s as a model strain for epilepsy research (Noda et al., 1998). These animals typically experience their first seizure as early as at 2 months of age; then, they exhibit spontaneous, generalized, tonic-clonic seizures. The typical seizure pattern of NERs appears as neck and forelimb clonus, followed by wild jumping and/or running, and finally by tonic convulsions followed by clonic convulsions. Ictal cortical electroencephalograms (EEGs) obtained during these spontaneous tonic-clonic seizures have revealed that the seizures commence with bilaterally synchronized small spikes and develop into high-voltage spikes, followed by polyspike-wave complexes concurrent with the appearance of tonic and clonic convulsive behaviors. Histopathological studies have not revealed any abnormal findings in the central nervous system of these animals. The clinicopathologic manifestations are reminiscent of human generalized tonic-clonic epilepsy (Noda et al., 1998), suggesting that NERs represent an animal model of primary generalized tonic-clonic epilepsy. Genetic studies have indicated that a major autosomal recessive mutant gene controls the inheritance of seizures in NERs, with modifications by several minor genes (Noda et al., 1998). Furthermore, two epilepsy-related loci, for which positive linkages have been reported, exist on chromosome 1 and on chromosome 3. However, the genetic basis of epilepsy in NERs is not yet fully understood (Maihara et al., 2000).

In spite of the indication that NERs represent a good model of generalized tonic-clonic epilepsy, responses to traditional AEDs in NERs have only been tested against audiogenic seizures induced by acoustic priming in juvenile animals (Iida et al., 1998; Sasa et al., 1997) or with the kindling procedure (Ishimaru et al., 2010). To date, NERs have not been used to test AED effectiveness against spontaneous seizures, without using previously primed animals. Spontaneous seizures occur relatively infrequently in these animals (1—5 times/month; unpublished data), making the assessment of AEDs against spontaneous seizures difficult. Moreover, juvenile NERs are more susceptible to the acoustic priming stimulation than normal juvenile rats. Audiogenic seizures in NERs can be evoked by weekly acoustic priming stimulation from 3 to 22 weeks of age (Iida et al., 1998; Sasa et al., 1997). Although such primed seizures facilitate the assessment of AEDs in cases with few seizures, these also lead to the formation of certain artificial neurocircuits in the NERs.

Adult NERs are also susceptible to environmental disruptions, such as bedding replacement or unpleasant sensory stimuli. However, they do not exhibit seizures after repeated presentation of the same unpleasant stimulus, indicating that environmental stimuli do not induce the development of new circuits, but evoke seizures through existing mechanisms. The aim of our study was to develop a method for screening drugs for use as anticonvulsants in the NER strain, which has infrequent seizures. Therefore, we evaluated traditional AEDs (phenobarbital [PB] and sodium valproate [VPA]) against non-artificially primed seizures in adult NERs, using strong annoying stimuli, rather than using animals primed during adolescence.

Materials and methods

Animals

Eighty male NERs were used in total. All NERs, derived from animals purchased from Japan SLC (Hamamatsu, Japan), were born and raised on-site. The animals were housed in groups of 3 in polymeric cages with contact bedding and were maintained on a 12-h light-dark cycle with white lights on from 7:00 pm to 7:00 am. Food (CE-2; CLEA Japan, Tokyo, Japan) and water were freely available.

Twenty-four animals were used for the PB experiments; the animals were divided into 3 groups (Groups 1, 2, and 3) of every eight animals. Sixteen animals were used for the VPA experiments; the animals were divided into 2 groups (Groups 4 and 5) similarly (Table 1A).

The animals were 5 months old at the time of their first drug administration. The rats weighed around 500 g. By the third or the 4th experiment, the weight of the rats had increased (Table 1A).

Forty rats were used for pharmacokinetic studies. Rats were divided into 2 groups (Group A and B). Group A was used for getting plasma concentration—time curves following daily drugs administration over 7 days. Rats of Group A were divided into four sub-groups of every 5 rats for administration of PB (2.5 mg/kg and 5 mg/kg) and VPA (50 mg/kg and 100 mg/kg). Group B was used for getting plasma concentration—time curves in a day. Rats of Group B was divided into sub-groups similarly to Group A (Table 1B).

All experimental procedures involving animals were approved by the Committee on Animal Experiments at Azabu University and were carried out in accordance with the Guidelines for Animal Experiments at Azabu University, Japanese Government Animal Protection and Management Law (No. 105) and the Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6).