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Administration of diazepam during status epilepticus reduces development and severity of epilepsy in rat

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Abstract

Prevention of epileptogenesis after brain insults, such as status epilepticus (SE), head trauma, or stroke, remains a challenge. Even if epilepsy cannot be prevented, it would be beneficial if the pathologic process could be modified to result in a less severe disease. We examined whether early discontinuation of SE reduces the risk of epilepsy or results in milder disease. Epileptogenesis was triggered with SE induced by electrical stimulation of the amygdala. Animals ($n = 72$) were treated with vehicle or diazepam (DZP, 20 mg/kg) 2 h or 3 h after the beginning of SE. Electrode-implanted non-stimulated rats served as controls for histology. All animals underwent continuous long-term video-electroencephalography monitoring 7–9 weeks and 11–15 weeks later to detect the occurrence and severity of spontaneous seizures. As another outcome measure, the severity of hippocampal damage was assessed in histologic sections. In the vehicle group, 94% of animals developed epilepsy. DZP treatment reduced the percentage of epileptic animals to 42% in the 2-h DZP group and to 71% in the 3-h DZP group ($p < 0.001$ and $p < 0.05$ compared to the vehicle group, respectively). If epilepsy developed, the seizures were less frequent in DZP-treated animals compared to the vehicle group (median 16.4 seizures/day), particularly in the 2-h DZP group (median 0.4 seizures/day). Finally, if DZP treatment was started 2 h, but not 3 h after SE, the severity of hippocampal cell loss was milder and the density of mossy-fiber sprouting was lower than in the vehicle group. These data indicate that treatment of SE with DZP within 2 h reduces the risk of epilepsy later in life, and if epilepsy develops, it is milder.

Keywords: Amygdala; Antiepileptogenesis; Disease modification; Mossy fiber sprouting; Prevention; Temporal lobe epilepsy; Video-EEG

1. Introduction

Status epilepticus (SE) is a major neurologic emergency occurring in approximately 0.1% of the

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population (Shorvon, 1994; DeLorenzo et al., 1996). The mortality associated with SE can be up to 50% and depends on the underlying etiology, age, duration of SE, and efficacy of treatment (for review, see Shorvon, 1994). Factors associated with morbidity, including epileptogenesis, drug-resistance, and cognitive decline, are less well identified. A recent epidemiologic study reported that up to 42% of individuals with status epilepticus as their first seizure develop epilepsy over the next 10 years (Hesdorffer et al., 1998). Risk factors for epileptogenesis include brain injury and prolonged duration (>60–90 min) of SE (Yager et al., 1988; Hesdorffer et al., 1998). A question remains whether treatment of SE affects epileptogenesis or, if the development of epilepsy cannot be completely blocked, if the severity of epilepsy be alleviated (i.e., disease-modifying effect).

Several experimental studies investigated the efficacy of antiepileptic drugs (AEDs) on SE-induced epileptogenesis by administering compounds during the epileptogenic phase (for a review, see Löscher, 2002; Pitkänen, 2002a,b; Pitkänen and Kubova, 2004). The AEDs tested include gabapentin (Cilio et al., 2001), levetiracetam (Klitgaard et al., 2001), phenobarbital (Mikati et al., 1994; Bolanos et al., 1998), topiramate (see Löscher, 2002), and valproate (Bolanos et al., 1998; Klitgaard et al., 2001). Some antiepileptogenic efficacy was reported with valproate, topiramate, and gabapentin. There are caveats, however, to interpreting the data. First, in many of these studies, animals were still receiving anticonvulsant doses of the AED when seizures were monitored (gabapentin: Cilio et al., 2001; phenobarbital: Mikati et al., 1994; phenobarbital and valproate: Bolanos et al., 1998). Second, seizure detection was based on a short-term video monitoring without long-term video-electroencephalography (EEG) recording to detect electrographic seizures (Cilio et al., 2001; Mikati et al., 1994; Bolanos et al., 1998). Third, only secondarily generalized seizures were counted (Cilio et al., 2001; Mikati et al., 1994; Bolanos et al., 1998), and therefore, the occurrence of partial seizures cannot be excluded. Many of these problems have been overcome by studies using long-term video-EEG monitoring. Klitgaard et al. (2001) investigated the antiepileptogenic effect of levetiracetam and valproate and Halonen et al. (2001) as well as André et al. (2001) investigated that of vigabatrin. No antiepileptogenic effects were reported. Therefore, based on the data available, there is no hard pre-clinical evidence that any of the AEDs in use would be antiepileptogenic when administered during a latency period. Also, no disease-modifying effects on the frequency, duration, or behavioral severity of seizures in rats who eventually developed epilepsy have been reported (phenobarbital: Mikati et al., 1994 and Bolanos et al., 1998; levetiracetam: Klitgaard et al., 2001; valproate: Klitgaard et al., 2001; vigabatrin: Halonen et al., 2001 and André et al., 2001). Similarly, clinical studies have failed to demonstrate antiepileptogenesis with AEDs (Temkin, 2001).

In previous studies, the AEDs were administered for several weeks during a latency period. Another strategy would be to interfere with the epileptogenic process at an earlier phase, for example during SE or within a few hours thereafter. Therefore, we analyzed our database of 72 long-term video-EEG monitored animals collected during the past 4 years to examine whether "initial insult modification", that is, shortening of the duration of SE, is antiepileptogenic. Even if the development of epilepsy cannot be prevented, perhaps the epilepsy that develops will be milder (disease modification). SE was induced by electrical stimulation of the amygdala (Nissinen et al., 2000). After 2 h or 3 h, SE was discontinued with diazepam (DZP). Antiepileptogenic and disease-modifying effects were assessed 2–3 months later using long-term video-EEG monitoring of spontaneous seizures (occurrence, frequency, duration, and behavioral severity) and histology.

2. Methods

Study design is summarized in Fig. 1.

2.1. Animals

Adult male Harlan Sprague–Dawley rats ($n = 155$; 275–390 g) were used in the study. Animals were individually housed in cages in a controlled environment (constant temperature, $22 \pm 1\,^{\circ}C$, humidity 50–60%, lights on 07:00–19:00 h) and had free access to food and water. All animal procedures were conducted in accordance with the guidelines set by the European Community Council Directives 86/609/EEC.

2.2. Induction of SE

Details of the methodology were recently published (Nissinen et al., 2000). Briefly, to induce SE via elec-