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The effect of the cannabinoid-receptor antagonist, SR141716, on the early stage of kainate-induced epileptogenesis in the adult rat

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Summary

Pre-treatment with the endocannabinoid-receptor antagonist, SR141716, has been reported to suppress the long-lasting hyperexcitability and increased seizure susceptibility present after 30 min of hyperthermia-induced convulsions in immature rats, an animal model of complex febrile seizures in children, which may be a cause of temporal lobe epilepsy. The present experiments tested the hypothesis that SR141716 suppresses epileptogenesis in the adult kainate model, an animal model of temporal lobe epilepsy. Adult male rats (n=35), implanted for electroencephalogram (EEG) recordings, were treated with kainate. Immediately after the first acute electrographic seizure during kainate-induced status epilepticus, either vehicle or SR141716 (10 mg/kg) was injected intraperitoneal. Chronic video-EEG data were collected for the first 2-week period after kainate-induced status epilepticus. Over half of *both* the vehicle- and drug-treated animals showed spontaneous recurrent seizures. Similarly, mean seizure frequency was not significantly different for the drug- and vehicle-treated animals during the first 2 weeks (n=9 and 8, respectively). Thus, no significant differences were found between SR141716-treated and control animals during the first 2 weeks of epileptogenesis. These results suggest that the endocannabinoid-receptor antagonist, SR141716, had no detectable effect on the early stages of epileptogenesis in the adult kainate model. Several potential explanations for the differences in the effects of SR141716 in the adult-rat, kainate versus immature-rat, hyperthermia models are discussed.

Keywords

epilepsy; febrile seizures; status epilepticus; seizure threshold

Introduction

The induction of prolonged (i.e., 30 min) hyperthermic convulsions by a heated airstream in postnatal day 10 rat pups has been used as an animal model of complex febrile seizures (Dube et al., 2007), and this treatment protocol has been reported to cause an increased seizure susceptibility (Dube et al., 2000) and to lead to spontaneous recurrent seizures (Dube et al., 2006). Several studies have reported that the increased hippocampal hyperexcitability

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associated with this model is associated with an increase in both GABA-mediated inhibition and an intrinsic, hyperpolarization-activated depolarizing current (I_h) (Chen et al., 1999, 2001).

The endocannabinoid-receptor antagonist SR141716 blocks depolarization-induced suppression of inhibition and is proconvulsive (Armstrong et al., 2009). Administration of SR141716 immediately before the induction of hyperthermic convulsions in rat pups (on postnatal day 10) reverses the subsequent increase in seizure susceptibility (present several weeks after the hyperthermic convulsions), determined as a decrease in the latency to and duration of acute kainate-induced seizures (Chen et al., 2003, 2007). Similar results have been obtained after lateral fluid percussion injury, an animal model of post-traumatic epilepsy (Echegoyen et al., 2009).

The development of new therapies to block epileptogenesis would benefit from practical yet quantitative analyses of how pharmacological treatments affect epileptogenesis (i.e., the development of spontaneous recurrent seizures). Previous data from our group have shown that the development of spontaneous recurrent seizures is a sigmoid function of time (i.e., fit by a Boltzman function) after kainate-induced status epilepticus, and suggest that the latent period may not be the best measure of epileptogenesis (Williams et al., 2009). The present paper reports a series of electrographic recording experiments that were designed to test the hypothesis that SR141716 reduces epileptogenesis in the kainate model of epileptogenesis in the adult rat. The preliminary data do not support the hypothesis; however, they lead to a discussion of the strengths and weaknesses of the two animal models and the different approaches for testing for therapy-induced changes in epileptogenesis.

Experimental approach and results

Adult Sprague-Dawley rats (n=35 males) were implanted for electroencephalogram (EEG) recordings, and were then treated with kainate (repeated low-dose protocol), as has been described previously (Williams et al., 2009). Within 1 min after the occurrence of the first, 30-sec electrographic seizure during kainate-induced status epilepticus, either vehicle (alcohol/saline 1:20 ratio) or SR141716 (10 mg/kg) was injected intraperitoneal. Chronic video-EEG data were collected for several 2-week periods that were separated by 6-week intervals. The present report provides data from the first 2-week period. Later time periods will require additional analyses, and will be reported in a subsequent manuscript.

Both vehicle-injected control and SR141716 drug-treated animals developed spontaneous recurrent seizures during the first 2 weeks after kainate-induced status epilepticus, and these seizures had properties characteristic of the kainate model (Fig. 1)(Dudek et al., 2006; Williams et al., 2009). The proportion of animals with spontaneous recurrent seizures during the 2-week period was not significantly different for the vehicle- versus SR141716-treated groups (i.e., 57% [8/14] for the vehicle-treated animals and 52% [9/17] for the drug-treated animals)(Fig. 2A1). During the first 2-week recording epoch, the average seizure frequency was also not significantly different for the two groups (0.71 ± 0.24 seizures/day [n=9] for the drug-treated animals and 0.39 ± 0.12 seizures/day [n=8] for the vehicle-treated animals)(Fig. 2A2). Thus, many kainate-treated rats from both groups developed spontaneous recurrent seizures within 2 weeks after kainate treatment, and no significant differences were found between SR141716-treated and control animals during the early stage of epileptogenesis (i.e., slow growth phase). We have continued to monitor these animals to test whether SR141716 alters the severity of fully-developed epilepsy, although data analyses are still in progress.

Discussion

These data suggest that treatment with the endocannabinoid-receptor antagonist, SR141716, does not affect the development of spontaneous recurrent seizures during the first 2-week period after kainate-induced status epilepticus in adult rats. Recordings from the control (kainate treatment *only*) and experimental (SR141716 treatment with kainate) groups were conducted for many months, although some animals died and/or lost their EEG recordings (video data are, however, available). Additional analyses are now necessary to determine if SR141716 has an effect that was too modest to detect during the first 2 weeks of epileptogenesis. Nonetheless, SR141716 did not appear to have any effect. Below we consider five possible reasons for the differences in the effects of SR141716 in the immature-rat, hyperthermia model (Chen et al., 2007) and the adult-rat, kainate model.

First, one possibility for the different effects of SR141716 on epileptogenesis in the two studies could have been differences in the time of administration of SR141716. The experiments of Chen et al. (2007) involved SR141716 administration either before or after induction of hyperthermic seizures. Since strategies for blocking epileptogenesis that involve administration of potential therapies *before* the brain insult (e.g., induction of repetitive seizures) would have little or no clinical value, we injected SR141716 at the onset of kainate-induced status epilepticus, which was essentially identical to the protocol used by Chen et al. (2007). Specifically, Chen and coworkers (2007) administered SR141716 at 2 min after the induction of hyperthermic seizures, and SR141716 was given within 1 min after the onset of kainate-induced status epilepticus in the present study. Therefore, potential differences in the timing of administration of the compound do not seem to have been a factor in the different outcomes of the two studies.

Second, it is possible that SR141716 specifically blocks a pro-epileptogenic effect of hyperthermia that is not active or relevant to kainate-induced seizures or epileptogenesis. For example, SR141716 reduces depolarization-induced suppression of inhibition, and this mechanism of disinhibition could be relevant to the sequelae of hyperthermic seizures but not kainate-induced status epilepticus in the rat. If so, then we might predict that SR141716 would prevent epileptogenesis induced by hyperthermic seizures in older animals; however, the hyperthermia-induced convulsions are reported to be an early-development, age-dependent model, so it may not be feasible to study an adult-rat model based on hyperthermia-induced convulsions.

Third, the effect of SR141716 may be developmentally regulated, and no longer active in the adult rat. If so, then we would predict that SR141716 would exhibit antiepileptogenic effects in other models of epileptogenic injury to the developing brain, such as hypoxic-ischemic injury (Kadam et al. 2010). However, similar results to those from the immature-rat, hyperthermic convulsion model have been obtained after lateral fluid percussion injury in adult rats, an animal model of post-traumatic epilepsy (Echegoyen et al., 2009). Thus, a developmental effect does not appear to be an explanation for the difference.

Fourth, there may be differences in the sequelae of hyperthermia and kainate-treatment in these models of brain injury, particularly the degree to which the two models generate spontaneous recurrent seizures. As with all animal models of acquired epilepsy, these models have their strengths and weaknesses. Both the airstream-induced hyperthermic model of febrile seizures and the animal model of injury-induced, acquired epilepsy based on kainate-mediated status epilepticus (i.e., the repeated, low-dose kainate model) are intended to model temporal lobe epilepsy. It is well established that the kainate model of drug-induced status epilepticus develops spontaneous recurrent seizures, but all of the models based on status epilepticus are variable, often appear to show a relatively short latent

period to the first spontaneous recurrent seizure, and often have substantial brain damage (including areas outside of the hippocampus). Plus, relatively few cases of acquired epilepsy in humans have a history of severe status epilepticus. It has been argued that hyperthermic seizures in immature animals represent an age-appropriate model of febrile seizures during childhood, leading to temporal lobe epilepsy (Dube et al., 2007). On the other hand, one concern is that the pathophysiological effects of exogenously induced hyperthermia are not the same as the effects of endogenous fever. Also, an unresolved question regarding the hyperthermia model is whether the animals actually develop chronic epilepsy (i.e., ultimately show spontaneous recurrent seizures). In early studies using electrographic recordings, no evidence of spontaneous recurrent seizures were observed, even during prolonged recording periods, although changes in seizure susceptibility were noted (Dube et al., 2000). Subsequent studies have reported spontaneous recurrent seizures in the hippocampus. However, it is difficult to differentiate the events reported to be electrographic seizures in the EEG data of those reports (Dube et al. 2006) from physiological hippocampal theta rhythm (Whishaw, 1972). Further, the illustrated events are quite unlike the epileptic seizures typical of temporal lobe epilepsy and kainate-induced epilepsy. Therefore, although animals that have experienced a 30-min period of hyperthermia-induced convulsions early in development appear to show a subsequent, long-lasting change in seizure susceptibility (thus suggesting long-term, pro-convulsant changes in the brain), they may well lack a necessary step in epileptogenesis required to develop chronic epilepsy (i.e., the presence of spontaneous recurrent seizures). Thus, the 30-min hyperthermia model may reduce seizure threshold, but not to the point that characterizes chronic epilepsy. If so, then the lack of effect of SR14176A in the kainate model may be due to the fact that the kainate model represents a more severe model of brain injury and subsequent epilepsy.

Fifth, the lack of effect of kainate in our study may be due to the different assays used to detect an effect. Our study differs from the other SR14176 studies in regard to the assay of the epileptogenesis. The evidence that administration of SR14176 before the hyperthermia-induced convulsions alters subsequent epileptogenesis is based on the subsequent response of the treated animals to kainate injections. The strength of the evidence that SR14176 treatment (at the time of the hyperthermic insult) suppresses subsequent epileptogenesis depends on the value of the use of (1) latency-to-first seizure, (2) duration of the longest seizure, and (3) the total-seizure duration to acute kainate-induced seizures (Figs. 6D,E, and F, respectively, of Chen et al., 2007). As illustrated in Figure 2B, however, the latency-to-first seizure from a 5 mg/kg injection of kainate in the present study was quite variable, and at least some normal animals in our study (i.e., had not yet been treated with vehicle or SR14176) had a latency-to-first seizure in <10 min, similar to that reported for animals that experienced hyperthermic convulsions at post-natal day 10 (see Fig. 6D of Chen et al., 2007). The data in Figure 6 from Chen and coworkers (2007) indicate that the longest-seizure duration (Fig. 6D) and the total-cumulative seizure duration (Fig. 6E) were virtually identical, so both panels essentially show the same measure and reflect the duration of kainate-induced status epilepticus (if status epilepticus is defined as ≥ 10 min). In our hands, at least, the duration of the seizure activity in normal animals in response to a 5-mg/kg injection of kainate is quite variable, which was the original rationale for the development of the multiple, low-dose protocol (Dudek et al., 2006). Furthermore, in addition to its variability, the duration of kainate-induced status epilepticus is highly dependent on how it is measured, since the EEG seizures and spikes that occur after kainate treatment can stop rather suddenly or gradually. Thus, although seizure susceptibility (as assessed by latency-to-first seizure and seizure duration to a kainate challenge) may be viewed as a surrogate marker for the spontaneous recurrent seizures that define epileptogenesis, measures of seizure susceptibility are both indirect and variable assays of epileptogenesis. On the other hand, the frequency and pattern of spontaneous recurrent seizures in the kainate model and

other models based on status epilepticus are also quite variable, but they are direct measures of epileptogenesis.

In conclusion, the data reported here suggest that administration of SR141716 before or during kainate-induced status epilepticus and subsequent brain injury in adult rats does not suppress epileptogenesis. Additional studies are warranted to further explore the hypothesis that administration of endocannabinoid antagonists modifies epileptogenesis in other epilepsy models, optimally employing an assay for epileptogenesis that includes the measurement of spontaneous recurrent seizure activity.

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Figure 1.

Spontaneous recurrent seizure after treatment with SR141716. Electrographic seizure recorded from a single surface-cortical electrode in an SR141716-treated rat with kainate-induced epilepsy. Note that the seizure was >30 sec, and had a characteristic onset including a seconds-long build-up and an abrupt termination. Three time points (1-3) are temporally expanded below.

Figure 2.

Measures of epileptogenesis and seizure susceptibility. **(A)** Effect of SR141716 on epileptogenesis during the first 2-week period after kainate-induced status epilepticus, based on the occurrence of spontaneous recurrent seizures. **(A1)** Percentage of vehicle- and SR141716-treated animals exhibiting seizures during the first 2-week period after kainate treatment. **(A2)** Seizure frequency of vehicle- and SR141716-treated animals with seizures during the first 2-week period after kainate treatment. Note that SR141716 had no detectable effect either the proportion of animals showing spontaneous recurrent seizures (A1) or on the frequency of spontaneous seizures (A2). **(B)** Variability in measurements of latency-to-first seizure in response to injections of kainate (5 mg/kg) in the present study. Before administration of vehicle or SR141716, the rats were injected with kainate; if a rat did not respond with a seizure(s), the rat was injected again with kainate, and all rats responded by the 5th injection. The five bars show the mean ($\pm$ SEM) latency-to-first seizure of the rats in the present study. The closed circles show the individual measures; note the variability of seizure latency in normal rats.