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Single Application of a CB1 Receptor Antagonist Rapidly Following Head Injury Prevents Long-Term Hyperexcitability in a Rat Model

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Abstract

Effective prophylaxis for post-traumatic epilepsy currently does not exist, and clinical trials using anticonvulsant drugs have yielded no long-term antiepileptogenic effects. We report that a single, rapid post-traumatic application of the proconvulsant cannabinoid type-1 receptor antagonist SR141716A (Rimonabant-- Acomplia®) abolishes the long-term increase in seizure susceptibility caused by head injury in rats. These results indicate that, paradoxically, a seizure-enhancing drug may disrupt the epileptogenic process if applied within a short therapeutic time window.

Keywords

Traumatic brain injury; Epilepsy; Rimonabant; CB1

1. Introduction

Successful intervention to prevent the development of late seizures (occurring a week or more) after brain injury remains one of the greatest unmet challenges facing epilepsy research (Garga and Lowenstein, 2006). The incidence of traumatic brain injury (TBI) is high (about 1 per 500 population per year), and accounts for 20% of symptomatic epilepsy in the general civilian population (Hauser et al., 1991). Several clinical trials have been conducted to test whether antiepileptic (anticonvulsant) drug prophylaxis reduces the risk of developing late (also called spontaneous, epileptic, unprovoked and remote symptomatic) seizures after TBI. These clinical trials, using the anticonvulsants phenobarbital, phenytoin, carbamazepine and valproate, showed beneficial effects restricted to early post-traumatic seizures, without any significant reduction in the incidence of late-onset seizures (i.e., post-traumatic epilepsy) (Garga and Lowenstein, 2006, Temkin, 2001). Significantly, not only has there been no positive outcome from the clinical trials, but there have also been no previous reports of successful prophylaxis for post-traumatic epilepsy with any drug, even in experimental animals. The failures of clinical trials and lack of evidence in animal experiments suggest that the focus for prophylaxis for post-traumatic epilepsy needs to shift from antiepileptic drugs to antiepileptogenic drugs, or in other words, from anticonvulsants effective in controlling acute seizures to drugs that block the inciting molecular events that lead to post-traumatic epileptogenesis. However, one cannot

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exclude the possibility that prophylaxis for post-traumatic epilepsy using post-injury drug application is not an achievable goal, as perhaps the injury immediately initiates events which rapidly become irreversible, resulting in epileptogenesis.

Although the molecular steps leading to epileptogenesis are not fully understood, there are some promising candidates that may be targeted for possible prophylaxis. Cannabinoid type-1 (CB1) receptor-mediated signaling, in particular, has been suggested to be mechanistically important in the acute control of seizures (Monory et al., 2006, Wallace et al., 2003). The endogenous cannabinoid ligands (endocannabinoids) for CB1 receptors are synthesized and released “on-demand” from postsynaptic neurons during heightened neuronal activity, including seizures, and CB1 receptors are present on both excitatory and inhibitory nerve terminals where they inhibit glutamate and GABA release, respectively (Katona and Freund, 2008). Because of their strategic position on excitatory terminals, CB1 receptor agonists are potent anticonvulsants (Monory et al., 2006, Wallace et al., 2003). Conversely, CB1 antagonists are proconvulsants in already hyperexcitable networks, such as in pilocarpine treated animals or tetanized tissue (Chen et al., 2007, Wallace et al., 2003). In addition to playing a role in acute seizures, the activity-dependent rise in endocannabinoids and the rapid downstream activation of CB1 receptors may also be important in triggering long-term hyperexcitability after an insult. Indeed, recent results showed that CB1 receptor blockade during experimental febrile seizures prevents the emergence of febrile seizure-induced long-term changes in seizure susceptibility (Chen et al., 2007, Lutz and Monory, 2008). There are considerable challenges associated with testing antiepileptogenic effects of CB1 antagonists on spontaneous seizures (see 3. Results & Discussion). Therefore, as a first step towards identifying potential post-traumatic antiepileptogenic effects of CB1 receptor antagonists, we tested the hypothesis that CB1 receptor blockade after TBI prevents the long-term increase in post-traumatic seizure susceptibility.

2. Methods

A major component of post-traumatic epilepsy is the persistent increase in seizure susceptibility that can be precisely measured and quantitatively compared by recording EEG under proconvulsant challenge (Kharatishvili et al., 2007). Therefore, we used the classic limbic proconvulsant, kainate to test the effect of CB1 receptor antagonism on long-term post-traumatic seizure susceptibility 6 weeks after TBI.

Moderate lateral fluid percussion injury (FPI) head trauma (2.0–2.2 atm) or sham FPI (in which animals were anesthetized and attached to the fluid percussion device, but the pendulum was not dropped) was performed on P21–22 Wistar rats (35–50g, Charles River, Wilmington, MA) as described previously (Howard et al., 2007, Santhakumar et al., 2000, Toth et al., 1997). Animals were divided into groups (see 3. Results & Discussion) receiving different drug treatments, administered only once via intraperitoneal (i.p.) injection. The CB1 receptor antagonist SR141716A (SR; trade name Rimonabant-- (Acomplia®); 1mg/kg or 10mg/kg) was first dissolved in alcohol, then added to normal saline (alcohol to saline ratio: 1:20). The composition and volume of the vehicle solution administered was identical, but SR was not added. SR was injected either immediately (within 2min) or 20min after injury. Pentobarbital (40mg/kg), when administered concurrently, was injected immediately after SR.

Seizure susceptibility to low-dose (5 mg/kg) kainate was tested 6 weeks later *in vivo* using hippocampal depth electrodes (single twisted-wire electrode; Plastics One, Roanoke, VA) placed stereotaxically in the ipsilateral dorsal CA1 region several days before recording. The EEG signal was processed as described previously (Chen et al., 2007). Baseline EEG activity was recorded for 10 min and 60min of continuous EEG monitoring followed kainate injection.

Seizures were accompanied by typical behavioral alterations (e.g. freezing and limbic automatisms) recorded by an observer, and EEG seizure criteria were as described previously (Chen et al., 2007): (1) repetitive spike and sharp-wave discharges at >1 Hz, (2) EEG amplitude >2x baseline, (3) duration >5s. Termination was defined as the absence of the first two criteria for >3s. Statistical analyses were performed using a one way ANOVA followed by a Dunnett's post hoc test with $p < 0.05$. Data are presented as mean $\pm$ SEM; n is the number of recorded animals.

3. Results & Discussion

In agreement with previous reports (Coulter et al., 1996, Kharatishvili et al., 2007, Lowenstein et al., 1992, Santhakumar et al., 2000), post-FPI animals showed a significant, long-term increase in susceptibility to kainate-induced seizures compared to age-matched, littermate controls. Specifically, application of kainate 6 weeks after injury in animals that had experienced FPI followed by vehicle injection within 2min of impact resulted in seizures that appeared significantly faster and lasted significantly longer than in sham-injury control, vehicle-injected animals (Fig. 1; compare CON+VEH with FPI+VEH; $n=6$ and $n=14$ animals, respectively; $p=0.022$ seizure latency, $p=0.024$ seizure duration). The CB1 receptor antagonist SR had no effect on controls (Fig. 1; compare CON+VEH with CON+SR; $n=8$ animals for CON+SR; $p=0.986$ seizure latency, $p=0.655$ seizure duration; vehicle/SR injection: within 2min after sham-injury). The key finding of this study is that, in contrast to vehicle-treated FPI animals, animals that experienced FPI followed by SR treatment (within 2min after impact) showed no evidence of a long-term increase in average seizure susceptibility compared to control animals (Fig. 1; compare CON+VEH and FPI+SR; $n=17$ for FPI+SR; $p=1.00$ seizure latency, $p=0.996$ seizure duration; SR was applied at 1mg/kg or 10mg/kg i.p., and, since there was no significant difference between the two doses, the data were combined). These data, for the first time, demonstrate that 1) it is possible to prevent the TBI-induced long-term increase in seizure susceptibility with post-traumatic application of a drug (as noted above, there has been no other drug of any kind that has been shown to prevent the emergence of persistently increased seizure susceptibility after head injury); 2) a single application of the CB1 receptor antagonist SR is sufficient to produce this effect; and 3) counter-intuitively, a proven proconvulsant drug can have significant effects on preventing long-term post-traumatic hyperexcitability.

The rationale for the early application of SR in the experiments described up to this point was that if CB1 receptor activation is important in triggering events leading to a persistent increase in seizure susceptibility, it may be critical to block CB1 receptors as soon as possible after injury. Indeed, subsequent experiments showed that there was no significant beneficial effect of SR when applied 20min after FPI (Fig. 2; compare FPI+SR with FPI+SR_{20min}; $n=5$ for FPI+SR_{20min}; $p=0.023$ seizure latency, $p=0.036$ seizure duration). Therefore, future antiepileptogenic interventions may need to be administered within a critical time window that is considerably shorter than what has been considered acceptable in prior clinical trials (several hours to days) (Garga and Lowenstein, 2006, Temkin, 2001). While a similarly short time window in humans would limit the clinical utility of the CB1 antagonist, it would not abolish it, as the drug could be made available for rapid application in situations where there is an increased risk of head trauma (e.g. combat zones or sporting events).

Although animal models can never fully represent the clinical condition, it is important to know whether negative results from clinical trials can be accurately repeated in animal models. Therefore, in a final series of control experiments, we tested whether an anticonvulsant drug such as pentobarbital fails to prevent the long-term, post-traumatic increase in limbic seizure susceptibility, paralleling the negative results from the various clinical trials that have tested anticonvulsants as prophylactics for post-traumatic epilepsy. Indeed, pentobarbital (PENT)

treatment (within 2min after FPI) showed no significant beneficial effect on post-traumatic seizure susceptibility (Fig. 2; compare FPI+SR with FPI+PENT; n=8 for FPI+PENT; p=0.012 seizure latency, p<0.001 seizure duration). Furthermore, co-application of pentobarbital with SR after FPI (within 2min) abolished the beneficial effect of SR (Fig. 2; compare FPI+SR with FPI+PENT+SR; n=5 for FPI+PENT+SR; p=0.047 seizure latency, p=0.042 seizure duration), indicating that anticonvulsant co-application may interfere with the ability of SR to prevent the development of the long-term increase in seizure susceptibility after TBI. Future experiments will be needed to determine the mechanism responsible for this interference (e.g. blockade of SR's proconvulsant effect or a direct interaction through the GABAergic system).

Taken together, these results demonstrate that it is possible to prevent the long-term increase in seizure susceptibility, a likely mechanistic component of post-traumatic epilepsy, with the one-time application of a CB1 receptor antagonist. Significantly, SR is a clinically approved anti-obesity drug in over 40 countries (Rimonabant) (Despres et al., 2005, Pi-Sunyer et al., 2006), and while prolonged use has raised concerns about neurological side-effects (e.g. depression & anxiety, with other neurological side-effects at extremely low incidence), our results indicate that a single dose may be sufficient to prevent post-traumatic hyperexcitability. Given the results in this paper indicating the potentially beneficial effects of SR, our results may now be followed up with experiments testing SR in various trauma and epilepsy paradigms on spontaneous seizure incidence and frequency. There are, however, at least two major challenges that will need to be overcome. First, most models of post-traumatic epilepsy have a low incidence and frequency of spontaneous seizures (0.3 seizures/day in (Kharatishvili et al., 2006; but see D'Ambrosio et al., 2004), making drug studies on spontaneous post-traumatic seizures impractical. Second, in models of epilepsy where the initiating event necessarily outlasts the observed brief (<20min) time window for the beneficial effect of SR described above (such as kindling or chemically induced epilepsy models), the proconvulsant nature of SR may interfere with the induction process (e.g., SR exacerbation of pilocarpine-induced seizures makes the comparison between the pilocarpine and pilocarpine + SR treatment groups ambiguous). Further, SR's narrow therapeutic time window and its pro-convulsant nature pose challenges to future clinical applications which will need to be investigated. In summary, the present data demonstrate that, paradoxically, drugs such as SR displaying proconvulsive actions should be considered for future study as antiepileptogenic prophylactic therapies (Pitkänen et al., 2004).

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Figure 1. Single post-traumatic application of the CB1 antagonist SR 141716A (SR) prevents the long-term increase in seizure susceptibility after brain trauma

Seizure susceptibility testing with kainate 6 weeks after fluid percussion injury (FPI) reveals increased seizure susceptibility in the head-injured, vehicle-treated (FPI+VEH) animals ($n=14$) compared to the sham-injured, vehicle-treated (CON+VEH) animals ($n=6$; $p=0.022$ seizure latency, $p=0.024$ seizure duration), but not in the head-injured, SR-treated (FPI+SR) animals ($n=17$; $p=1.00$ seizure latency, $p=0.996$ seizure duration; SR injection within 2min after FPI). Note that SR injection (2min after sham injury) had no effect on the control animals (CON+SR; $n=8$; $p=0.986$ seizure latency, $p=0.655$ seizure duration). **(a)** Representative traces from hippocampal depth EEG recordings made during baseline recording or 5 min after injection of kainate. **(b)** FPI results in a significant decrease in seizure latency, and this decrease is eliminated by post-traumatic injection of SR. **(c)** FPI results in a significant increase in the cumulative duration of the seizures, and this increase is eliminated by post-traumatic injection of SR. Asterisks indicate significant difference from CON+VEH using a one way ANOVA followed by a Dunnett's post hoc test with a level of significance of $p < 0.05$. Error bars: SEM.

Figure 2. Narrow therapeutic time window for beneficial effects of SR on long-term seizure susceptibility and lack of beneficial effect of the anticonvulsant pentobarbital

(a, b) Seizure susceptibility testing with kainate 6 weeks after FPI (as in Fig. 1) reveals that, in contrast to rapid (within 2min) post-traumatic application of SR (FPI+SR; n=17; same data as in Fig. 1, shown here for comparison), delayed (20min after injury) application of SR (FPI+SR_{20min}; n=5) has no beneficial effect on seizure latency (panel a; p=0.023) and total seizure duration (panel b; p=0.036). In addition, pentobarbital administration (FPI+PENT; n=8) within 2min after injury does not prevent long-term increase in seizure susceptibility, as evidenced by decreased seizure latency (a; p=0.012) and increased total seizure time in (b; p<0.001). Finally, pentobarbital does interfere with the beneficial action of SR when co-applied within 2min after injury (FPI+PENT+SR; n=5; p=0.047 seizure latency, p=0.042 seizure duration). Note that FPI+PENT+SR is significantly different from FPI+SR, but is similar to FPI+VEH illustrated in Fig. 1. Asterisks indicate significant difference from FPI+SR using a one way ANOVA followed by a Dunnett's post hoc test with a level of significance of p < 0.05. Error bars: SEM.