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Neuromodulatory role of endogenous interleukin-1 β in acute seizures: possible contribution of cyclooxygenase-2

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Abstract

The function of endogenous interleukin-1 β (IL-1 β) signaling in acute seizure activity was examined using transgenic mice harboring targeted deletions in the genes for either IL-1 β (*Il1b*) or its signaling receptor (*Il1r1*). Acute epileptic seizure activity was modeled using two mechanistically distinct chemoconvulsants, kainic acid (KA) and pentylenetetrazole (PTZ). KA-induced seizure activity was more severe in homozygous null (-/-) *Il1b* mice compared to their wild-type (+/+) littermate controls, as indicated by an increase in the incidence of sustained generalized convulsive seizure activity. In the PTZ seizure model, the incidence of acute convulsive seizures was increased in both *Il1b* and *Il1r1* -/- mice compared to their respective +/- littermate controls. Interestingly, the selective cyclooxygenase (COX)-2 inhibitor, rofecoxib, mimicked the effect of IL-1 β deficiency on PTZ-induced convulsions in *Il1r1* +/- but not -/- mice. Together, these results suggest that endogenous IL-1 β possesses anticonvulsive properties that may be mediated by arachidonic acid metabolites derived from the catalytic action of COX-2.

Keywords

Interleukin-1 β ; Interleukin-1 receptor type 1; epilepsy; acute seizures; pentylenetetrazole; kainic acid; knockout mice; cyclooxygenase-2; rofecoxib

Introduction

Interleukin-1 β (IL-1 β) was initially characterized as a macrophage/monocyte-derived cytokine that possessed T lymphocyte stimulatory and proinflammatory properties (Farrar et al., 1980). However, IL-1 β mRNA and protein are now known to be expressed by numerous other cell types, suggesting a broader function beyond the immune system. In the normal central nervous system (CNS), for example, all components of the IL-1 signal transduction system appear to be present (Farrar et al., 1987; Breder et al., 1988; Takao et al., 1990; Quan et al., 1996; Loddick et al., 1997; Schneider et al., 1998; French et al., 1999; Hammond et al., 1999; Huitinga et al., 2000; Toyooka et al., 2003). Moreover, several studies have provided evidence for diurnal or rapid, activity-dependent release of IL-1 β from neurons (Tringali et al., 1996; Tringali et al., 1997; Watt and Hobbs, 2000; Hailer et al., 2005). These observations raised the possibility that IL-1 β may act in a neuromodulatory capacity in the

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healthy CNS (Vitkovic et al., 2000). In support of this notion, compelling evidence suggests a physiological role for IL-1 β in non-rapid eye movement sleep (Taishi et al., 1997; Krueger et al., 2001; Imeri and Opp, 2009) and cognition (Schneider et al., 1998; Avital et al., 2003; Ross et al., 2003; Goshen et al., 2007; Spulber et al., 2009b).

In addition to its physiological activities in the normal CNS, IL-1 β has been implicated in the pathologic processes associated with various CNS maladies (Fogal and Hewett, 2008; Shafteel et al., 2008; Rijkers et al., 2009). Relevant to the current report, evidence from several studies suggests that IL-1 β may influence acute temporal lobe seizure activity. However, the nature of its role remains controversial. While some evidence suggests that endogenous IL-1 β possesses pro-convulsant properties in acute seizure paradigms (Vezzani et al., 1999; Plata-Salaman et al., 2000; Vezzani et al., 2000; Vezzani et al., 2002; Heida and Pittman, 2005; Ravizza et al., 2008), other results are consistent with an acute anticonvulsive function of IL-1 β (Miller et al., 1991; Sayyah et al., 2005). Hence, further examination of this putative modulator of seizure activity is warranted.

In the present study, the effect of endogenous IL-1 β on seizure activity was investigated using two different transgenic mouse lines, which have targeted disruptions in genes for either the ligand, IL-1 β (*Il1b*), or its signaling receptor, IL-1 receptor type I (*Il1r1*). Cyclooxygenase-2 (COX-2), a key enzyme in the metabolism of arachidonic acid to potent autocrine and paracrine eicosanoid mediators, is an important effector of IL-1 β responses (Dinarello, 2002) and some actions of IL-1 β in the CNS have been linked to products of COX-2 (Terao et al., 1998; Inoue et al., 1999; Samad et al., 2001; Ferri and Ferguson, 2005; Sang et al., 2005). Because COX-2 has been implicated in acute seizure activity (Baik et al., 1999; Kunz and Oliw, 2001; Kim et al., 2008), the possible interplay between endogenous IL-1 β and COX-2 activity was also investigated.

Materials and Methods

Animal Husbandry

All mice were housed on a 12hr light/dark schedule (7am/7pm) in the Center for Laboratory Animal Care at the University of Connecticut Health Center, which is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Standard mouse chow and water were provided *ad libitum*. Animal procedures were conducted in accordance with the National Institute of Health guidelines for the use of experimental animals and were approved by the University of Connecticut Health Center's Institutional Animal Care and Use Committee.

Colonies of homozygous mutant (-/-) *Il1b* (Zheng et al., 1995) and *Il1r1* (Glaccum et al., 1997) mice were established from breeders obtained from Taconic Farms, Inc. (Model # 000612-M with a mixed background of B6B10R3129, Hudson, NY) and The Jackson Laboratories (Stock #003245 with the incipient congenic background of C57BL/6, Bar Harbor, ME), respectively. All genotyping was performed via PCR analysis of tail genomic DNA samples using gene-specific primer sets (Supplemental Data, Table 1). Male 8-12 week old wild-type and mutant littermates for studies were derived from heterozygous (+/-) breeding units (F₁) that were obtained by crossing -/- male mice from each mutant line with wild-type (++) female C57BL/6 mice (Stock #000664, The Jackson Laboratories, Bar Harbor, ME). F₂ +/- progeny were also used as breeding units for studies. Upon retiring these F₂ breeding units, new F₁ +/- breeder sets were derived from the -/- colonies as described above. This breeding strategy and the use of littermate controls was employed to control for potential, non-specific genetic influences or environmental differences (Pick and Little, 1965; Wolfer and Lipp, 2000; Wolfer et al., 2002). All mice were acclimated to handling by performing mock daily i.p. injections for one week prior to each study.

Mice were treated with KA or PTZ without knowledge of genotype. Littermates for KA studies were initially genotyped at weaning to remove heterozygous mice, which were not assessed in this model. The $+/+$ and $-/-$ mice were housed 3-4 per cage, ensuring that members of each genotype were represented in each cage. These mice were re-genotyped at the conclusion of the KA studies. For studies with PTZ, littermates including heterozygous mice were housed randomly 3-5 per cage and genotyped upon conclusion of each study.

Dosing Protocols and Seizure Scoring

Injection solutions of KA (Cayman Chemical Co., Ann Arbor, MI) were prepared in 0.05M PBS immediately prior to use and sterilized by filtration. Acute seizure activity was elicited in mice using a modified repeated low-dose KA treatment paradigm (Hellier et al., 1998). The paradigm was initiated with a loading dose of 20 mg/kg KA (10mL/kg, i.p.) followed by booster doses of 5 mg/kg KA administered 30, 60, 90, and 150 min later. To reduce mortality, seizure activity was terminated 240 min after initiation of the paradigm by administration of 5 mg/kg diazepam (0.9% saline containing 40% propylene glycol, i.p.). Mice were monitored continuously over the 240 min KA dosing paradigm by an observer blinded to genotype and KA-induced behavioral seizure activity was scored using the following scale: normal behavior (0); hypomobility and hypoactivity (1); episodic myoclonus (2); sustained sitting posture with kyphosis and episodic forelimb clonus (3); isolated generalized convulsive seizures without loss of righting reflex (4); isolated generalized convulsive seizures with loss of righting reflex (5); sustained generalized convulsive seizure activity (6). The maximal seizure score for individual mice was assigned to each 10 min interval of the 240 min paradigm (i.e., 24 intervals). The scores for each genotype group were compiled within each of the 24 intervals and these were referred to as the median maximal seizure score (MMSS). Latencies to discrete events, such as first convulsive seizure and mortality, were rounded to the ten minute interval in which the event occurred. For analysis purposes, the KA treatment paradigm was also subdivided into 60 min quarters (Q1-4).

PTZ (Sigma Chemical Co., St Louis, MO) was dissolved in 0.9% saline, filter sterilized, and administered intraperitoneally (i.p.) in a volume of 6.7mL/kg. Based on results from a dose-response analysis performed in wild-type C57BL/6 (Supplemental Data, Figure 1), acute seizure activity was induced by a single dose of 36 or 43 mg/kg PTZ. In one study, mice were treated with 30 mg/kg rofecoxib (p.o.) or its vehicle, 0.5% carboxymethylcellulose (CMC), 3 hrs prior to treatment with PTZ. Rofecoxib is an orally-active and highly selective inhibitor of COX-2 that is permeable to the blood-brain barrier (Chan et al., 1999; Ehrich et al., 1999; Bingham et al., 2005; Dembo et al., 2005). The rofecoxib dosing paradigm was chosen to maximize *in vivo* efficacy (Dembo et al., 2005; Hewett et al., 2006). A lower dose of PTZ (32 mg/kg) was used in this study to assess potentiation of seizure activity by COX-2 inhibition. Mice were monitored for 15 minutes after PTZ administration and seizure behavior scored using a modified Racine scale (Racine, 1972; Ferraro et al., 1999) as follows: 0, no behavioral change; 1, hypoactivity; 2, at least two isolated, myoclonic jerks; 3, generalized clonic convulsions, with preservation of righting reflex; 4, generalized clonic or tonic-clonic convulsions with loss of righting reflex. To ensure unbiased scoring, all seizures were scored by an observer blinded to genotype. In addition to seizure behavior, convulsion latency (time to onset of convulsive behavior, score ≥ 3) and incidence (% of mice exhibiting convulsions) were also quantified.

Electroencephalographic (EEG) Recordings

During the KA treatment paradigm, brain electrical activity was monitored in three freely mobile mice from each genotype using a small animal radio-telemetry system from Data Sciences International (DSI, St. Paul, MN). The surgical procedure employed was adapted

from previous studies using similar equipment (Kramer and Kinter, 2003; Bastlund et al., 2004; Krestel et al., 2004; Weiergraber et al., 2005). Mice were deeply anesthetized using 400 mg/kg chloral hydrate (i.p.) and restrained with a stereotaxic frame (Model 900, Kopf Instruments, Tujunga, CA). Temperature was monitored and maintained using a rectal temperature probe and homeothermic blanket system (Harvard Apparatus, Holliston, MA). The scalp was shaved and sterilized with betadine and a small mid-line incision was made with a sterile scalpel to expose the skull. Two 0.9-1.0mm deep burr holes were drilled through the bone at -1.5 mm bregma and 1.5 mm laterally using a high power dental drill attached to the stereotaxic frame apparatus. Stainless steel screws (00-90, 3/32" oval fillister, J.I. Morris Co., Southbridge, MA) were fitted into the burr holes and the leads from the magnetically-activated small animal radio-telemetry transmitter (TA10EA-F20, DSI) were attached. The screws and leads were covered with freshly prepared dental acrylic (Stoelting Co., Wood Dale, IL) and the transmitter was placed in a dorsal subcutaneous pouch and affixed to the skin using a single stitch. Following the closure of the incision, mice were placed in heated cages and administered a single dose of buprenorphine (0.05 mg/kg in 0.9% NaCl, s.c.) after resolution of anesthesia. Mice were singly-housed thereafter and allowed to recover from surgery for 10 days prior to KA administration.

On the day of KA administration, the cages were placed on the radio-telemetry receiver plates (Model RPC-1, DSI) that were hardwired to a dedicated computer via a Data Exchange Matrix (DSI) and baseline EEG traces were acquired at 1 kHz using Dataquest A.R.T. software (version 4.1, DSI). After 1 hr of baseline recordings, mice were administered KA using the repeated low-dose paradigm and seizure severity was scored as described above while EEG patterns were under constant telemetric surveillance. EEG recordings were digitally stored and analyzed off-line. The entire 240 min trace of EEG activity for each mouse was visually inspected by an observer blind to genotype and the predominant EEG pattern type (i.e., ≥ 30 sec) was assigned to each 1 min interval (see Results for description of EEG pattern types). EEG patterns were classified based on previous descriptions (Bragin et al., 1999; Riban et al., 2002; McColl et al., 2003; Arabadzisz et al., 2005; O'Sullivan et al., 2008).

Statistical Analysis

Behavioral data are reported as the median seizure scores alone or incidence of generalized convulsions (%). All statistical analyses were performed using GraphPad Prism (Version 4.03, GraphPad Software, Inc.) except for the 2×3 Fisher's exact test, which was performed using an on-line statistical tool (Uitenbroek, D.G. Simple Interactive Statistical Analysis, <http://www.quantitativeskills.com/sisa/>). Specific tests are indicated within the text of the "Results" section and/or legends to Figures. Statistical significance was set at $p < 0.05$.

Results

Higher Incidence of Sustained Generalized Convulsive Seizure Behavior and Mortality in *Il1b*^{-/-} mice treated with KA

The selective glutamate receptor agonist, KA, is a chemoconvulsant that has been widely used to model temporal lobe seizures (Robinson and Deadwyler, 1981; Westbrook and Lothman, 1983; Sharma et al., 2007; Vincent and Mulle, 2009). Since initial attempts to induce seizure behavior using a bolus KA dosing paradigm yielded inconsistent results and was accompanied by high mortality in C57BL/6 wild-type mice (data not shown), a repeated low-dose treatment paradigm was developed and employed herein (Figure 1). In this paradigm, an initial loading dose of 20 mg/kg KA was followed by 5 mg/kg booster doses given 30, 60, 90, and 150 min later to attain a cumulative dose of 40 mg/kg. All seizure activity was terminated with diazepam 240 min after the KA loading dose.

All mice exhibited similar seizure behavioral responses during the first 2 hr period of the dosing paradigm regardless of genotype (Q1-2, Figure 1). This was characterized by an initial profound decrease in spontaneous locomotor activity (KA seizure score = 1), which was followed by a gradual increase in the KA median maximal seizure score (MMSS), such that by the end of Q2, 100% (17/17) of *Il1b* *+/+* and 95% (21/22) of *-/-* mice had experienced at least one generalized convulsive seizure (KA seizure score ≥ 4). The latency to onset of convulsive seizure activity was not statistically different between genotypes (median time to first convulsive seizure following administration of the loading dose of KA was 60 and 50 min in *Il1b* *+/+* and *-/-* littermates, respectively; $p = 0.20$, Mann-Whitney test). Moreover, the MMSS of the two genotype groups peaked at a similar level of severity (KA seizure score > 4) upon administration of the final booster dose of KA at 150 min of the treatment paradigm. Thereafter, although the seizure behavior was sustained in both genotypes until it was terminated with diazepam at 240 min of the treatment paradigm, the KA responsiveness between the two genotypes appeared to differ qualitatively during this period (Q3-4, Figure 1). Thus, whereas the MMSS of the *Il1b* *-/-* genotype group persisted at ≥ 5 , the MMSS of the *Il1b* *+/+* genotype group moderated to 3. However, the value at 240 min in the latter group did not differ statistically from the respective peak MMSS at 150 min ($p > 0.05$, Friedman test with Dunn's multiple comparison test). Additionally, the MMSS values for the two genotype groups at 240 min were not statistically different from each other ($p = 0.092$, two tailed Mann-Whitney test). It is important to note, however, that mice that did not survive this treatment paradigm were not included in the analysis of these data (see below).

The behavioral response progressed to a condition of sustained generalized convulsive seizure activity (KA seizure score = 6) in a fraction of *Il1b* *+/+* and *-/-* mice subjected to the repeated low-dose KA treatment paradigm. Although the time to onset of this response did not differ between the two genotypes (latency was 125 ± 12 and 135 ± 14 min, median $\pm$ SEM, for *Il1b* *+/+* and *-/-* mice respectively; $p = 0.9115$, Mann-Whitney test), the incidence in *Il1b* *-/-* genotype group was >2 -fold higher than the *Il1b* *+/+* control group (Figure 2A). Additionally, in spite of the repeated low-dose KA treatment paradigm, mortality nevertheless remained a risk. Thus, a total of 1 of 17 (6%) and 7 of 22 (32%) mice succumbed within 3 days of the dosing paradigm in the *+/+* and *-/-* genotype groups, respectively (Figure 2B). Importantly, 5 mice in the *Il1b* *-/-* group were lost during the 240 min dosing paradigm and hence were excluded from the analysis of seizure responses in Figure 1. Since only one *Il1b* *+/+* mouse was excluded, this selective elimination of *Il1b* *-/-* mice with high seizure scores could have contributed to the lack of statistical significance demonstrated in the progression of MMSS in Figure 1.

KA-induced Changes in Brain Electrical Activity Paralleled Behavioral Responses in *Il1b* *+/+* and *-/-* mice

In addition to behavioral changes, KA-induced alterations in brain electrical activity were monitored in a cohort of *Il1b* *+/+* and *-/-* littermates (Figure 3). These mice were fitted with EEG-monitoring electrodes and changes in brain electrical activity were recorded over the entire 240 min course of the KA dosing paradigm (Figure 1). It is important to note that the behavioral responses of electrode-fitted *Il1b* *+/+* and *-/-* mice progressed similarly to non-electrode fitted mice of the corresponding genotype, thus indicating that the electrode implantation *per se* did not alter the differential sensitivity to KA (Supplemental Data, Figure 2). Each 240 min EEG trace from individual *Il1b* *+/+* and *-/-* mice (Figure 3A) were examined in 1 min intervals, revealing activity patterns that could be classified into 4 basic types based on their predominant association with distinct behavioral responses (Figure 3B). Type 1 activity was characterized by low amplitude (≤ 0.2 mV) noise without evident synchrony. This activity, which did not differ from the baseline activity observed prior to KA treatment, was typical of KA-induced hypomobility and myoclonus (KA seizure score =

1-2). Type 2 activity was characterized by 1-2 Hz spike and waves and was associated predominantly with seizure behavior that was characterized by periods of kyphosis and episodes of focal clonus (KA seizure score = 3). Type 3 activity included a mix of low frequency (≤ 0.5 Hz) spikes, 1-2 Hz spike and waves, and high frequency (≥ 4 Hz) spikes with ripples, while the Type 4 activity pattern contained low frequency spikes with fast ripples. Type 3 or Type 4 activity patterns were associated exclusively with generalized convulsive seizure behavior (KA seizure score ≥ 4).

The total duration of each EEG activity pattern (Type 1-4) was summed for *Il1b* $+/+$ and $-/-$ mice during each quarter (Q1-4) of the 4 hr KA-dosing protocol (Figure 3C). Regardless of genotype, as the KA dosing protocol progressed through Q1-2, the duration of the non-convulsive EEG activity patterns (Types 1 and 2) decreased, while activity patterns associated with convulsive seizures (Types 3 and 4) increased. This is consistent with the onset of convulsive behavior and progressive elevation of the MMSS observed in each genotype during the first half of the KA dosing paradigm (Figure 1). During the last two hours of the paradigm (Q3-4), the duration of the types of EEG activity patterns that correlated with convulsions was greater in *Il1b* $-/-$ mice compared to *Il1b* $+/+$ littermates, although this difference was not statistically significant (Friedman test, $p > 0.05$). Nevertheless, this result paralleled qualitatively the difference in the behavioral responses between the two genotypes during this period (Figure 1).

Increased Incidence of PTZ-induced Convulsions in Mice Lacking IL-1 β Signaling

The neuromodulatory role of endogenous IL-1 β in acute seizures was explored further in the PTZ model of acute seizures. PTZ is a GABA_A receptor antagonist that has been used extensively to model generalized epileptic seizure activity and to test the antiepileptic properties of drugs (Ticku and Ramanjaneyulu, 1984; White, 1997; Huang et al., 2001). The acute behavioral response to treatment with 36 mg/kg PTZ, a dose approximating the median effective dose in wild-type C57BL/6J mice (Supplemental Data, Figure 1), was qualitatively similar in either *Il1b* or *Il1r1* mutant mouse lines regardless of the genotype of progeny. An initial profound decrease in locomotor activity (PTZ seizure score = 1) was often followed by sporadic myoclonic seizures (PTZ seizure score = 2). In a fraction of the mice, this behavior evolved into a solitary generalized convulsive seizure (PTZ seizure score ≥ 3), which resolved spontaneously within approximately ten seconds of onset. All acute behavioral manifestations of seizures occurred within fifteen minutes of PTZ administration and animals resumed normal activity after a brief postictal period of inactivity. PTZ treatment did not induce sustained seizure activity, fatalities, or hippocampal neurodegeneration in any genotype group (data not shown).

The heterozygous ($+/-$) and homozygous ($-/-$) genotype groups from the *Il1b* mutant line exhibited median PTZ seizure severity scores of 2.5 and 4.0, respectively, in response to 36 mg/kg PTZ (Figure 4A). However, these responses were not statistically different from wild-type ($+/+$) littermate controls, which had a median PTZ seizure score of 1.5. In agreement with this result, 36 mg/kg PTZ elicited a median PTZ seizure severity score of 2.0 in all genotype groups from the *Il1r1* mutant mouse line (Figure 5A). Moreover, the latency to convulsions did not differ between genotypes of either line (data not shown). Interestingly, however, further analysis of the data revealed an increase in the incidence of convulsive seizures in mutant *Il1b* and *Il1r1* mice. Although this increase was not statistically significant in mutant *Il1b* mice ($p = 0.0963$; χ^2 test for independence), a significant linear trend across genotypes was detected in this mouse line ($p = 0.0435$; χ^2 test for trend) (Figure 4B). On the other hand, the incidence of convulsive seizures was significantly increased in mutant *Il1r1* mice (Figure 5B). The difference in incidence between genotypes of the *Il1r1* line was dose-dependent, as it was not detected at 43 mg/kg

PTZ (incidence of convulsions = 11/12 [92%] and 9/11 [82%] for *Il1r1* *+/+* and *-/-*, respectively).

Absence of Rofecoxib Effect on Acute PTZ-induced Seizures in *Il1r1* *-/-* mice

Seizures elicited by NMDA are more severe in COX-2 *-/-* mice (Toscano et al., 2008) and COX-2 inhibitors, including rofecoxib, can exacerbate seizure activity induced by PTZ (Claycomb and J.A. Hewett, unpublished observation) and KA (Baik et al., 1999; Kunz and Oliw, 2001; Kim et al., 2008). The enhancement of acute seizure activity by COX-2 inhibition was recapitulated herein using *+/+* mice from the mutant *Il1r1* mouse line. Systemic PTZ (32 mg/kg) elicited convulsive seizures in 10% (1/10) of wild-type mice pretreated with CMC vehicle, while pretreatment with 30 mg/kg rofecoxib increased the incidence to 56% (5/9) as anticipated (Figure 6). This five-fold increase in the incidence of convulsive seizures was remarkably similar to the incidence of PTZ-induced convulsions in *Il1r1* *-/-* mice pretreated in parallel with CMC (55% or 6/11) (Figure 6). This similarity between the effects of COX-2 inhibition and IL-1R1 deficiency, together with the well-documented effector role of COX-2 in IL-1 β signaling, raised the possibility that the altered PTZ responsiveness of the *Il1r1* *-/-* genotype observed in Figure 6 might result from a consequent loss of COX-2 activation in these mice. If so, it was posited that rofecoxib would not further enhance the incidence in *Il1r1* *-/-* mice. Indeed, the incidence of convulsive seizures in *Il1r1* *-/-* mice pretreated with rofecoxib was 43% (6/14) which was not statistically different from CMC-treated *Il1r1* *-/-* mice ($p = 0.430$, one-tailed Fisher's exact test).

Discussion

The results in this report are consistent with the conclusion that disruption of endogenous IL-1 β signaling renders mice more prone to acute convulsive seizure activity. It is interesting to note that while KA-induced seizure activity in both *Il1b* *-/-* and *+/+* mice progressed with similar onset and severity prior to 150 min in the repeated low-dose treatment paradigm, sustained generalized convulsive seizure activity and mortality were exacerbated in the mutant cohorts. These results raise the intriguing possibility that IL-1 β may serve as an endogenous termination mechanism in this model of sustained seizure activity. Consistent with this notion, IL-1 β expression is induced within various brain regions 2-3 hrs after systemic or focal administration of KA (Yabuuchi et al., 1993; Vezzani et al., 1999), an effect that has been linked to excitatory neuronal activity (Eriksson et al., 2000). A number of endogenous anticonvulsant mechanisms have been implicated in the spontaneous resolution of seizure activity (Lado and Moshe, 2008). Of these, adenosine has been linked to the IL-1 β -induced inhibition of synaptic transmission in the hippocampus (Luk et al., 1999). Thus, it is possible that IL-1 β functions to suppress or limit the hyperexcitability of persistent convulsive seizure activity via an adenosine-dependent mechanism. This hypothesis warrants further investigation.

In the PTZ model of generalized seizure activity, the incidence of convulsive seizures was increased in IL-1 β signaling deficient mice without a significant change in latency to convulsions or median seizure severity scores. Moreover, PTZ-induced convulsive seizure activity in mutant mice resolved spontaneously in a manner that was indistinguishable from wild-type littermates. Hence, disruption of IL-1 β signaling did not result in sustained or multiple convulsions following PTZ administration. Together, these results suggest that lack of IL-1 β signaling results in a reduction in PTZ seizure threshold without affecting the rate of generalization or termination.

The molecular mechanism underlying these anti-epileptic properties of endogenous IL-1 β remains to be elucidated fully. However, the similarity between the altered PTZ sensitivity

observed in *Il1r1* $-/-$ mice and rofecoxib-treated wild-type mice raises the possibility that the reduced threshold for convulsions in the absence of intact IL-1 signaling may be due to a loss of COX-2 activation. Further support for this was provided by the observation that, in contrast to wild-type littermates, rofecoxib did not additionally increase the sensitivity of *Il1r1* $-/-$ mice to the convulsant actions of PTZ. These results are consistent with evidence linking COX-2 to the CNS actions of IL-1 β in several instances. For example, exogenous IL-1 β failed to induce fever in COX-2 null mice or in mice treated with selective COX-2 inhibitors (Li et al., 2001). Other examples include the influence of IL-1 β in CNS control of neuroimmune response, slow-wave sleep, and pain hypersensitivity (Terao et al., 1998; Samad et al., 2001; Ferri and Ferguson, 2005).

An effector role of COX-2 in the anti-epileptic actions of IL-1 β in the PTZ model presupposes that COX-2 activity is rapidly augmented in the CNS by acute seizure activity and that metabolites of COX-2 possess anti-convulsive properties. Unlike most cells outside of the CNS, COX-2 is constitutively expressed by excitatory neurons (Yamagata et al., 1993; Breder et al., 1995; Adams et al., 1996; Joseph et al., 2006), particularly within the cerebral cortex and hippocampus, and prostaglandin production is indeed augmented in these brain regions following PTZ exposure (Berchtold-Kanz et al., 1981; Forstermann et al., 1982; Engelhardt et al., 1996). Moreover, a time-course analysis indicated that this increase precedes convulsive seizure behavior and studies with KA and electrical stimulation demonstrated that elevated brain prostaglandin levels can persist for tens of minutes following the convulsive stimulus (Forstermann et al., 1982; Baran et al., 1987; Yoshikawa et al., 2006; Kim et al., 2008). Thus, COX-dependent arachidonic acid metabolism is a general initial and prolonged response of the brain to convulsive stimuli. Importantly, PGD₂, PGE₂, or PGF_{2 α} , the three most prominent brain prostaglandins elicited by convulsive stimuli, have each been shown to possess anti-convulsive properties in a variety of acute seizure models (Rosenkranz and Killam, 1981; Forstermann et al., 1983; Bhattacharya and Parmar, 1987; Akarsu et al., 1998; Kim et al., 2008).

Together, these results are consistent with the working hypothesis that endogenous IL-1 β may function to antagonize acute seizure activity via COX-2-dependent anti-convulsant prostaglandin production. Considering the short latency to convulsions in the PTZ model and the potential for rapid induction of arachidonic acid release from cells by IL-1 β (McHowat and Liu, 1997), it is reasonable to posit that IL-1 β may be linked to constitutive COX-2 via activation of phospholipase A₂ in this model. On the other hand, induction of new COX-2 protein expression may contribute to IL-1 β neuromodulation in the KA model, wherein the time to onset of convulsive seizure activity was much more extended. Pertinent to this, expression of COX-2 is induced by IL-1 β in many cell types, including neurons (Samad et al., 2001). In any case, further studies will be necessary to elucidate the details of the cellular and molecular mechanisms involved in this putative link between IL-1 β and COX-2 in these models of acute seizure activity.

It should be noted that the role of COX-2 in models of epileptic seizures has been somewhat controversial (Akula et al., 2008; Oliveira et al., 2008a; Oliveira et al., 2008b). Although the basis of this controversy remains unclear, the reduction in PTZ-induced seizure threshold reported herein in wild-type C57BL/6 mice treated with rofecoxib has been replicated in CD-1 mice (Claycomb and J.A. Hewett, unpublished results) indicating that it is not unique to the C57BL/6 background. It is also important to note that, contrary to the results from the present study, evidence from several previous reports concluded that IL-1 β facilitates seizure activity (Vezzani et al., 1999; De Simoni et al., 2000; Vezzani et al., 2000; Vezzani et al., 2002; Vezzani et al., 2004; Dube et al., 2005; Ravizza et al., 2006; Ravizza and Vezzani, 2006; Ravizza et al., 2008b). Although the reasons for this latter discrepancy are also not clear, it may be related to differences in experimental paradigms. For example, the

route of administration of convulsant stimuli may influence whether IL-1 β is pro- or anti-convulsive. In support of this idea, it has been demonstrated that IL-1 β facilitates seizure activity when both convulsant stimuli and IL-1 are administered locally into the hippocampus (Vezzani et al., 1999; De Simoni et al., 2000). Conversely, IL-1 β administration suppresses seizure activity when convulsants are delivered systemically (Miller et al., 1991). Further, the neuromodulatory actions of IL-1 β have been described to differ between brain regions. Thus, intracerebroventricular administration of IL-1 β exhibits anti-convulsant actions against seizures elicited by electrical stimulation of the amygdala (Sayyah et al., 2005), but pro-convulsant properties when electrical seizures are initiated in the hippocampus (De Simoni et al., 2000). Finally, the nature of the CNS actions of IL-1 β appear to depend on its concentration in the brain (Ross et al., 2003).

Although the alterations in seizure susceptibility reported herein could conceivably result from genetic background differences unrelated to the targeted gene mutations (Eisener-Dorman et al., 2009), this possibility seems unlikely given that similar results in the PTZ model were obtained from mice harboring targeted deletion of either the IL-1 β ligand (*Il1b*) or its signaling receptor (*Il1r1*). It is relevant in this regard that the targeted genes reside on separate mouse chromosomes (chr 2 and 1, respectively; Mouse Genome Informatics, <http://www.informatics.jax.org/>) and that the two mutations were maintained in different mouse strains (i.e., in different genetic backgrounds). Thus, corroboration of the results in these separate lines strengthens the contention that IL-1 β may indeed be an endogenous anticonvulsant neuromodulator.

Given that acute convulsions occurred within 5-10 minutes of PTZ exposure, results from this model imply that IL-1 β is expressed constitutively within the CNS and signals rapidly to affect seizure threshold. Pertinent to this supposition, IL-1 β expression has been reported under basal conditions in certain brain regions (Breder et al., 1988; Lechan et al., 1990; Huitinga et al., 2000) and some evidence suggests that it can be rapidly released from neurons in an activity-dependent manner (Tringali et al., 1996; Tringali et al., 1997; Watt and Hobbs, 2000; Hailer et al., 2005). On the other hand, since both of the mutant lines were derived using a traditional global gene-targeting approach, it remains possible that the altered seizure susceptibility phenotype of these mice could be consequent to the absence of the targeted genes during development (Eisener-Dorman et al., 2009). In this regard, IL-1 β expression has been reported in the postnatal CNS where it could modulate certain aspects of CNS development (Spulber et al., 2009a), including neural precursor cell proliferation and gliogenesis (Giulian et al., 1988; Wang et al., 2007). Further studies using a conditional gene-targeting approach will be necessary to rule out this possibility.

In summary, although commonly known for its role in acute inflammation, it is clear that IL-1 β can modulate neuronal activity in the CNS under both normal and pathophysiological conditions. Results in the current report provide compelling evidence for an anti-convulsive property of endogenous IL-1 β and shed new light on the apparent contradictory results of previous studies. Further understanding of the molecular mechanisms of these effects may facilitate development of novel therapeutic strategies to affect epileptic seizure activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- *Il1b* gene inactivation increases severity of kainate-induced seizures
- *Il1b* or *Il1r1* gene inactivation increases the incidence of PTZ-induced convulsive seizures.
- IL-1 β deficiency mimicked COX-2 inhibition.
- Endogenous IL-1 β possesses anticonvulsive properties that may be mediated by COX-2

Figure 1. Effect of *Il1b* gene-disruption on KA-induced seizure activity

Seizure activity was induced in *Il1b* +/+ and -/- littermates (n = 17 and 22, respectively) using a repeated low-dose KA treatment paradigm (“Materials and Methods”). Seizure behavior was scored on a 0-6 scale of increasing severity (see “Materials and Methods”). **A)** *Time-course of seizure behavioral response.* Results are expressed as the median maximal seizure score (MMSS) for each 10 min interval of the 240 min treatment paradigm (see materials and methods). The gray shaded times on the abscissa indicate the time of administration of the corresponding KA dose (mg/kg) indicated directly below across the bottom. Diazepam (5 mg/kg) was administered 240 min following the initial KA loading dose at 0 min. For descriptive and analytical purposes, the KA dosing paradigm is divided

into 60 min quarters (Q1-4, vertical dashed lines). **B) Individual seizure behavioral responses.** Points represent the maximal seizure score for each +/+ and -/- mouse during the first (10 min), 15th (150 min) and final (240 min) interval of the treatment paradigm in **A**. Note that mortality during the treatment paradigm resulted in the loss of one +/+ and five -/- mice from data in the latter time intervals (See Figure 5B). *, statistically different from the respective initial 10 min interval ($p < 0.05$, Friedman's test with Dunn's multiple comparison test on data in Figure 1A). No statistically significant differences between genotypes were detected.

Figure 2. Effect of *Il1b* gene disruption on sustained generalized convulsive seizure activity and survival after KA

A) Incidence of sustained generalized convulsive seizure activity. The number of *Il1b* +/+ and -/- mice that experienced KA seizure score = 6 in Figure 1 is expressed as the % of total number subjected to the KA dosing paradigm (fraction within bars). *, significantly different from +/+ group ($p = 0.0264$, Fisher's exact test). **B)** % survival. Kaplan–Meier survival curves up to 72 hrs following the KA dosing paradigm for *Il1b* +/+ and -/- mice from Figure 1. The gray box along the abscissa indicates the 240 min KA treatment paradigm. The two curves are significantly different ($p = 0.0488$, Mantel-Cox log-rank test).

Figure 3. Effect of *Il1b* gene-disruption on KA-induced brain electrical activity

Three *Il1b* $+/+$ and $-/-$ littermates ($N = 3$ each) were fitted with radio-telemetry transmitters and brain electrical activity was monitored in the motile mice as described in “Materials and Methods”. Each animal was subjected to the low-dose KA dosing paradigm as described in Figure 1. **A)** Gross KA-induced changes in brain electrical activity. EEG traces from individual $+/+$ (left column) and $-/-$ (right column) mice are shown for the entire 240 min KA exposure period. The gray shaded portion of each trace demarcates 30 min of baseline activity that immediately preceded the initial KA loading dose. Inset: amplitude (mV, ordinate) and time (min, abscissa). **B)** Representative traces of four common EEG pattern types. See text for detailed descriptions. Inset: amplitude (mV, ordinate) and time (sec,

abscissa). **C) Incidence of four common EEG pattern types.** A predominant EEG pattern type from **B** was assigned to each minute of the EEG traces in **A** and the % of time occupied by each type is depicted for each 60 min quarter (Q1-4) of the KA dosing paradigm (Figure 1). Differences between genotypes in the incidence of patterns associated with generalized convulsions (types 3 and 4) were not statistically significant ($p > 0.05$, Friedman's test).

Figure 4. Effect of *Il1b* gene-disruption on PTZ-induced seizure activity

Acute seizure activity was induced by PTZ (36 mg/kg, i.p.) in heterozygous (+/-) and homozygous (-/-) *Il1b* mutant mice or their wild-type (+/+) littermate controls and seizure behavior was scored on a 5 point scale of increasing severity as described in “Materials and Methods”. **A**) *Seizure severity*. Each point represents the maximum seizure score for individual mice. Results are from 6 separate experiments performed over 4 months. The median seizure scores for each genotype (horizontal line) are not statistically different ($p = 0.113$, Kruskal-Wallis one-way ANOVA). **B**) *Incidence of convulsions*. The number of mice exhibiting a convulsive seizure (PTZ seizure score ≥ 3) in **A** is expressed as a % of total mice exposed to PTZ for each genotype group (fraction within bars).

Figure 5. Effect of *Il1r1* gene-disruption on PTZ-induced seizure activity
Mice from each genotype were treated and scored as described in Figure 4. **A)** *Seizure severity*. Each point represents the maximum seizure score for individual mice. Results are from 4 separate experiments performed over 6 months. The median seizure scores for each genotype (horizontal line) are not statistically different ($p = 0.156$, Kruskal-Wallis one-way ANOVA). **B)** *Incidence of convulsions*. The number of mice exhibiting a convulsive seizure (PTZ seizure score ≥ 3) in **A** is expressed as a % of total mice exposed to PTZ for each genotype (fraction within bars). The incidence of convulsive seizures is statistically different across genotypes ($p = 0.0343$; two-tailed, 2×3 Fisher's exact test).

Figure 6. The pro-convulsive effect of rofecoxib is absent in mice lacking IL-1R1

Separate cohorts of mice from each genotype were treated with rofecoxib (30 mg/kg, p.o.) or CMC vehicle 3 hr prior to administration of PTZ (32 mg/kg, i.p.) and seizure behavior was scored as described in "Materials and Methods". **A)** Seizure scores for individual mice. **B)** The number of mice exhibiting a convulsive seizure (PTZ seizure score ≥ 3) is expressed as a % of total (fraction within bars). The difference in incidence of convulsions between CMC and rofecoxib treatments for each genotype was compared using a one-tailed Fisher's exact test. *, significantly different from the respective CMC treatment group ($p = 0.0495$).