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Lipopolysaccharide enhances glutaric acid-induced seizure susceptibility in rat pups: Behavioral and electroencephalographic approach

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Summary Glutaric acidemia type I (GA-I) is an inherited metabolic disease characterized by accumulation of glutaric acid (GA) and seizures. Considering that seizures are precipitated by common infections in children with GA-I, we investigated whether lipopolysaccharide (LPS) modifies GA-induced electrographic and neurochemical alterations in 21 days-old rats. The effect of LPS on convulsive behavior and electroencephalographic (EEG) alterations induced by GA (0.13; 0.4; 1.3 μmol/striatum) was determined in freely moving rats. After EEG recordings, we measured the levels of interleukin 1β (IL-1β) in GA-injected striatum. The injection of LPS (2 mg/kg; i.p.) 6 h before of GA administration, reduced the latency and increased the

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duration of seizures induced by GA (1.3 μmol/site). In addition, LPS administration increased IL-1β striatal levels, which positively correlated with total time in seizures. The intrastriatal injection of an IL-1β antibody (200 ng/2 μl) prevented the facilitation of GA-induced seizures by LPS. These data suggest that inflammatory processes during critical periods of development may decrease GA-induced seizure threshold.

Glutaric acidemia type I (GA-I) is an inherited neurodegenerative disease caused by deficiency of the mitochondrial enzyme glutaryl-CoA dehydrogenase (GCDH; EC 1.3.99.7), which is involved in the metabolism of L-lysine, L-hydroxylysine and L-tryptophan. Affected patients accumulate majorly glutaric acid (GA), 3-hydroxyglutaric acid (3-OH-GA) and glutaconic acid in the body fluids (Goodman et al., 1977). Clinical manifestations of GA-I are predominantly neurological, including convulsions, especially after encephalophatic crises, which are accompanied by bilateral and irreversible destruction of susceptible brain regions, i.e. striatum (Hoffmann and Zschocke, 1999). Furthermore, neurological crises are typically precipitated by common infection, febrile illness and after routine immunization, suggesting a role of inflammatory mediators in this organic aciduria (Hoffmann and Zschocke, 1999). A substantial body of evidence indicates that brief systemic inflammation during critical periods of development may result in long-lasting cerebral and peripheral vulnerability (Hagberg and Mallard, 2005). For instance, it has been shown that rats injected at postnatal day 14 (P14) with LPS are more susceptible to seizures induced by lithium–pilocarpine, kainic acid (KA) and pentylenetetrazole (PTZ; Galic et al., 2008).

The inflammatory response brought about by LPS is characterized by innate immune system activation and production of proinflammatory mediators, such as tumor necrosis factor (TNF) and IL-1β (Cohen, 2002). These proinflammatory cytokines are now accepted as bona fide modulators of both normal and abnormal neuronal transmission within the brain (Merrill, 1992).

In this context, previous reports have shown that GA-I metabolites interfere with astrocytic glutamatergic transporters (Porciúncula et al., 2004) and provide evidence that astrocyte proliferation is associated with mitochondrial dysfunction and oxidative stress (Olivera et al., 2008). In line of this view, the appearance of such newborn astrocytes may underlie the subsequent development of gliosis that disrupt brain development, perhaps contributing for the establishment of neurological deficits in GA-I patients (Goodman and Frerman, 2001). In addition, it has been shown that postnatal LPS administration results in a chronic though mild form of astrogliosis, a feature commonly found in a number of seizure models (Somera-Molina et al., 2007; Oberheim et al., 2008) and patients with epilepsy (Eid et al., 2008).

Although clinical and experimental evidence suggest that infection or inflammation facilitates seizure predisposition (Vezzani and Granata, 2005; Auvin et al., 2010; Vezzani and Baram, 2007) the pathogenesis of GA-induced convulsive behavior is still unknown. Therefore, we decided to investigate the involvement of proinflammatory cytokines during critical periods of development (Galic et al., 2008) in electroencephalographic and neurochemical alterations induced by GA. For this purpose we investigated the effect of LPS administration on seizures and alterations of striatal levels of IL-1β induced by GA in rat pups (21 days-old).

Experimental procedures

Animal and reagents

Twenty one days-old male Wistar rats (30–40 g), maintained under controlled light and environment (12:12 h light–dark cycle, 24 ± 1 °C, 55% relative humidity) with free access to food (Guabi, Santa Maria, Brazil) were used. Animal utilization protocols followed the Official Government Ethics guidelines and were approved by the University Ethics Committee. All efforts were made to reduce the number of animals used, as well as to minimize their suffering. All reagents were purchased from Sigma (St. Louis, MO).

Surgical procedure and behavioral evaluation

The animals were anesthetized with ketamine–xylazine (45–9 mg/kg body weight; i.p.) and placed in a rodent stereotaxic apparatus. Under stereotaxic guidance, a cannula was inserted unilaterally into the right striatum (coordinates relative to bregma: AP 0 mm, ML 3.0 mm, V 2.5 mm from the dura). Chloramphenicol (200 mg/kg) was administrated intraperitoneally (i.p.) immediately before the surgical procedure. The behavioral evaluation was performed 3 days after surgery when animals did not show any sign of pain, infection or discomfort.

In order to determine the convulsant dose of GA in rat pups, the animals were injected with GA (0.13; 0.4 or 1.3 μmol/2 μl) or saline (1.3 μmol/2 μl) into the right striatum. Glutaric acid solutions were neutralized with NaOH to pH 7.4 and injections were performed over a 1 min interval. Immediately after the intrastriatal injections, the animals were transferred to a round open field (54.7 cm in diameter) with a floor divided into 10 equal areas. During 20 min, the animals were videomonitored for the appearance of seizures. The latency to clonic and generalized convulsions and time in seizures were recorded. Clonic convulsions were characterized by typical partial clonic activity affecting the face, head, vibrissae and forelimbs. Generalized convulsive episodes were characterized by generalized whole-body clonus involving all four limbs and tail, rearing, wild running and jumping, sudden loss of upright posture and autonomic signs, such as hypersalivation and defecation, respectively.

To investigate whether inflammation alters seizure activity induced by GA, LPS (2 mg/kg; E. coli 055 B5; Eriksson et al., 2000; Maeda et al., 2008) or vehicle was infused i.p. (single administration) 3 or 6 h before the administration of GA (0.13; 0.4 or 1.3 μmol/site) or saline (1.3 μmol/site). The animals were then transferred to a round open field and convulsive behavior was evaluated as described above. Immediately after the behavioral evaluation, the animals were sacrificed by decapitation to determine IL-1β levels in the injected striatum.

Since LPS administration 6 h before GA intrastriatal injection facilitated convulsions and increased IL-1β levels, we investigated whether this cytokine is involved LPS-induced facilitation of GA-induced seizures. The animals were subjected to intrastriatal injection of IL-1β antibody (200 ng/2 μl) or IL-1β denatured antibody 45 min before injection of LPS (2 mg/kg; i.p.) or vehicle

(2 mg/kg, i.p.). This dose of IL-1β antibody was chosen because it neither caused seizures nor altered motor activity in a pilot experiment. Six hours after LPS or vehicle injection, GA (0.13, 0.4 or 1.3 μmol/site) or saline (1.3 μmol/site) was administrated and the appearance of seizures monitored for 20 min by behavioral and electroencephalographic recordings (EEG).

Placement of cannula and electrodes for EEG recordings

A subset of animals ($n = 5–6$) was anesthetized with ketamine–xylazine and surgically implanted with a cannula and electrodes under stereotaxic guidance. The guide cannula (27 gauge) was glued to a multipin socket and inserted into the right striatum through a previously opened skull orifice. One screw electrode was placed over the right parietal cortex (coordinates in mm: AP −4.5; ML 2.5; and V 2) along with a ground lead positioned over the nasal sinus. Bipolar nichrome wire Teflon-insulated depth electrodes (100 μm) were implanted into the right striatum (coordinates in mm: AP 0; ML 3; V 2.5). The electrodes were connected to the multipin socket and, together with the injection cannula, were fixed to the skull with dental acrylic cement. Electroencephalographic recordings were performed 3 days after the surgery.

Intrastriatal injection of drugs and EEG recording

The procedures for EEG recordings were carried out as previously described by Magni et al. (2007). Routinely, the animals were allowed to settle for habituation in a plexiglas cage (25 cm × 25 cm × 60 cm) for at least 20 min. Baseline EEG recordings were obtained 10 min prior to drugs administration in order to establish an adequate control brain electrical activity. The drug injection protocol used in this set of experiments was the same used in those experiments that evaluated the effect of LPS on GA-induced behavioral seizures, except that EEG was concomitantly recorded using digital encephalographic equipment (Neuromap EQSA260, Neuromap LTDA, Brazil). During 20 min the animals were videomonitored for the appearance of clonic or tonic seizures. The latency to seizures and time in seizures were recorded.

EEG signals were amplified, filtered (0.1–70.0 Hz, bandpass), digitalized (sampling rate 250 Hz) and stored in a PC for off-line analysis, as described below.

EEG analyses

Seizures were defined by the occurrence of episodes consisting of a pattern of continuous high-amplitude sharp spikes in low-frequency rhythmic spiking activity in ipsilateral striatum and cortex (≥2× baseline amplitude, ≥5 s). Handling for drug injection caused artifacts in the EEG (Figs. 1, 3 and 7). However, rhythmic scratching of the electrode headset by the animal rarely caused artifacts. These recordings were easily identified and discarded.

Rectal temperature measurement

In order to investigate the effect of infection state on the basal rectal temperature (T_r) of 21-days old rats, vehicle or LPS was infused intraperitoneally and T_r changes were recorded every hour for 3 or 6 h, and expressed as absolute value.

Rectal temperature was measured with a lubricated thermocouple, which was inserted into the rectum of the animal, for 1 min. The probe was linked to a digital device, which displayed the temperature at the tip of the probe with a 0.1 °C precision. The values displayed were manually recorded. In order to minimize the effects of the stress associated with handling and injecting on rectal temperature, all rats were habituated to the measuring procedure for two consecutive days. In these sessions, the animals were subjected to the same temperature measuring procedure described above.

IL-1β immunoassay

The content of IL-1β was determined in injected striatum (right). After the behavioral evaluation of seizures, the injected striatum was dissected rapidly out at 4 °C and frozen at −70 °C. The striatum was weighed and homogenized in a solution containing bovine serum albumin (BSA 10 mg/ml), EGTA (2 mM), EDTA (2 mM) and PMSF (0.2 mM) in phosphate-buffered saline (PBS, pH 7.4) using a Potter homogenizer. The striatum homogenate was centrifuged (3000 × g for 10 min) and cytokines were determined in supernatant. Cytokine levels were measured using a commercially available ELISA Kit from R&D Systems (Minneapolis, MN) using a selective antibody against rat IL-1β, according to the manufacturer's protocol, and are expressed as pg/mg of protein (detection limit: 4 ng/ml).

Protein determination

Protein content was measured colorimetrically by the method of Bradford (1976) using bovine serum albumin (1 mg/ml) as a standard.

Statistics

The behavioral data were analyzed by non-parametric test (Kruskal–Wallis analysis of variance). Data from IL-1β levels and rectal temperature measurements were analyzed by a two-way ANOVA. Post hoc analysis was carried out by the Student–Newman–Keuls test. $P < 0.05$ was considered significant. The Pearson's correlation coefficient was used for correlation analyses.

Results

GA-induced seizures

In the present study, the intrastiatal injection saline (1.3 μmol/site) and GA (0.13 μmol/site) did not induces convulsive behavior. On the other hand, statistical analysis revealed that intrastriatal injection of GA (1.3 μmol/site) decreased the latency to the first convulsive episode [164.88 ± 18.18 s; $H(3) = 24.08$, $P < 0.01$; Fig. 1F] and increased time in seizures 40.88 ± 4.5 s; [$H(3) = 25.58$; $P < 0.01$; Fig. 1G] as compared to GA (0.4 μmol/site) group. The EEG recordings confirmed these behavioral alterations elicited by the intrastriatal injection of GA (Figs. 1A–D). The electroencephalographically recorded GA-induced seizures were behaviorally accompanied by clonus of the left forelimb and/or hind limb and head, rotational behavior and full lateralization toward the left side of the body. In addition, EEG recordings of the ispilateral striatum revealed that the GA administration caused the appearance of an epileptogenic focus in the injected striatum that spread to the ipsilateral cortex. The EEG seizure activities were characterized by the occurrence of multispikes and major seizure activity. Multispikes correlated with myoclonic jerks, which are characteristic of clonic convulsions. Generalized convulsions appeared in the electroencephalographic recordings as the major seizure activity, and were characterized by 2–3 Hz high-amplitude activity (Figs. 1D–E). The animals that were

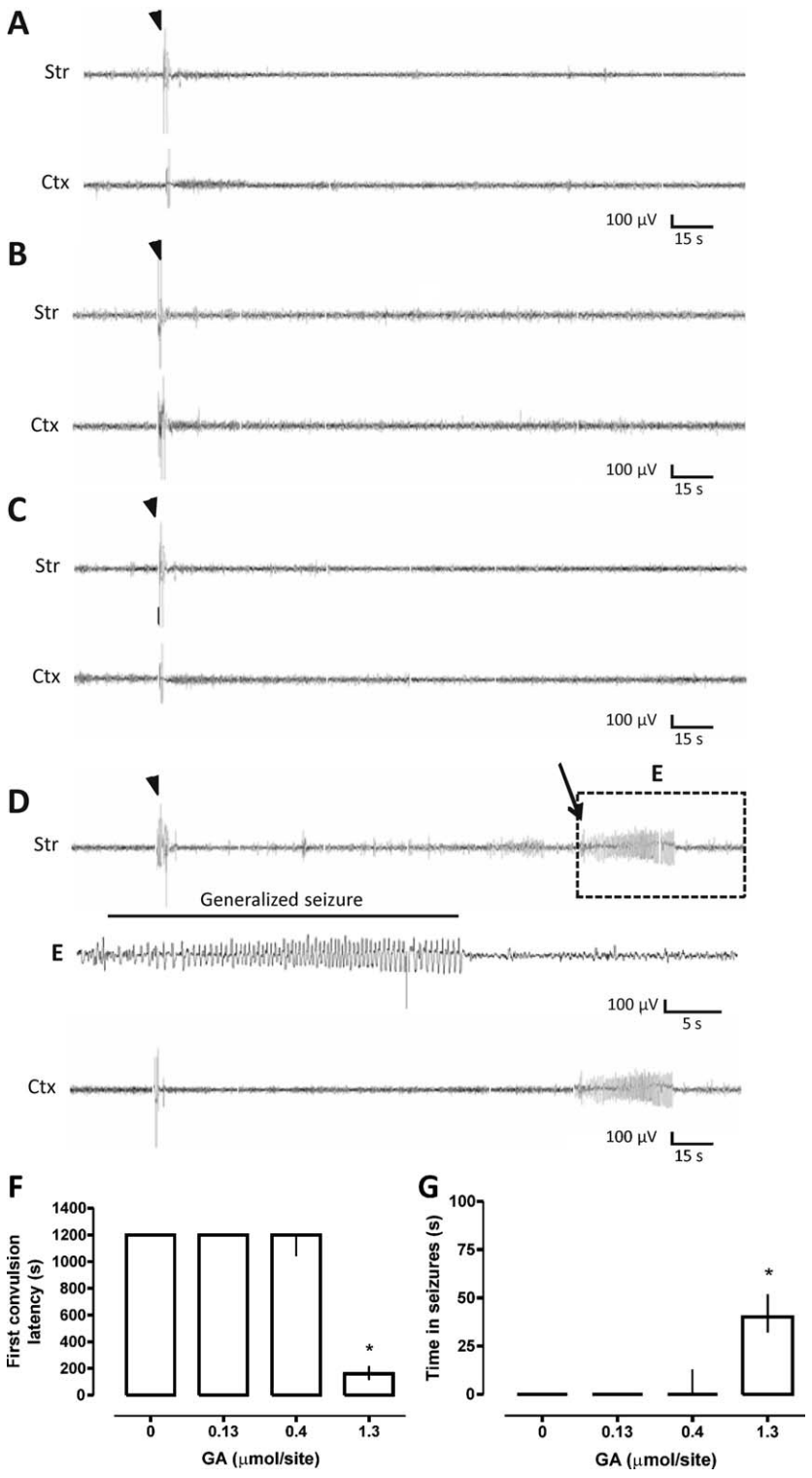

Figure 1 Representative electroencephalographic recordings: intrastriatal administration of saline (control; A), intrastriatal administration of GA (0.13 μmol/site; B), (0.4 μmol/site; C), and (1.3 μmol/site; D) which were accompanied by pattern of continuous high-amplitude sharp spikes in low-frequency rhythmic spiking activity in ipsilateral striatum and cortex. (E) The expanded waveforms from box of the EEG recording in D. In all traces the arrowhead indicates the saline or GA intrastriatal administration; the arrow indicates the EEG pattern associated with generalized seizures. Str, ipsilateral striatum; Ctx, ipsilateral cortex. Calibration bars, 100 μV and 15 s. Effect of intrastriatal injection of GA (0.13; 0.4; 1.3 μmol/site) on the first convulsion latency (F) and time in seizures (G) in rat pups. Data are median and interquartile range for $n = 6–8$ in each group.*$P < 0.01$ as compared to GA (0.4 μmol/site) group; Kruskal—Wallis analysis of variance.

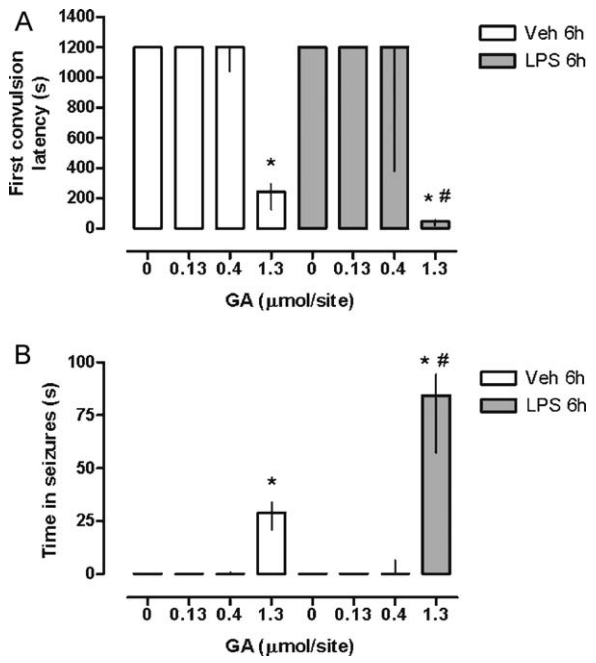

Figure 2 Effect of injection LPS (i.p.) 6 h before of the intrastriatal administration of GA (0.13; 0.4; 1.3 μmol/site) on the first convulsion latency (A) and time in seizures (B) in rat pups. Data are median and interquartile range for $n = 6-8$ in each group. $^{*}P < 0.05$ when compared to vehicle 6 h or LPS 6 h/GA (0.4 μmol/site) groups (A, B); $^{\#}P < 0.05$ when compared to vehicle 6 h/GA (1.3 μmol/site) group (A, B); Kruskal—Wallis analysis of variance.

injected with GA (1.3 μmol/site) showed 3.0 ± 0.55 seizures during the 20 min period of observation.

LPS facilitates GA-induced seizures

Statistical analysis revealed that administration of LPS 3 h before the intrastriatal injection of GA (all doses) had no effect on the latency to convulsions [119.62 ± 53.61 s; $H(7) = 5.46$; $P > 0.05$] and time in seizures [28.75 ± 8.04 s; $H(7) = 7.66$; $P > 0.05$] as compared to vehicle 3 h/GA (1.3 μmol/site) group (data not shown). On the other hand, statistical analysis revealed that the administration of LPS 6 h before intrastriatal injection of GA (1.3 μmol/site), decreased the latency to convulsions [39.7 ± 6.69 s; $H(7) = 66.54$; $P < 0.05$; Fig. 2A], and increased the time in seizures [80.7 ± 7.17 s; $H(7) = 66.05$; $P < 0.05$; Fig. 2B] as compared to vehicle 6 h/GA (1.3 μmol/site) group. Prior administration of LPS (2 mg/kg) did not cause behavioral changes.

LPS (2 mg/kg) injection did not affect baseline (248.21 ± 5.40 μV) or interictal mean amplitude, when compared with control group (234.88 ± 1.16 μV, $n = 6-8$ in each group; data not shown). EEG recordings showed that animals injected with LPS 3 h before GA injection (Fig. 3C) did not present significant alterations in EEG recordings when compared with the group that received LPS 6 h before GA injection (Fig. 3E). However, the injection of LPS 6 h before GA decreased the latency to seizures [38.55 ± 7.37 s; $H(2) = 7.05$; $P < 0.05$; Fig. 3G] and increased the time in seizures [67.33 ± 10.82 s; $H(2) = 10.97$; $P < 0.05$; Fig. 3H] as compared to vehicle/GA (1.3 μmol/site; Fig. 3A) and LPS 3 h/GA (1.3 μmol/site; Fig. 3C) groups. Furthermore, LPS (2 mg/kg) injection did not alter the number of convulsive episodes induced by GA (1.3 μmol/site, data not shown). No animals died due to GA-induced convulsions.

Effect of LPS on basal rectal temperature

LPS causes an initial drop in basal temperature (1—2 h) followed by a temperature increase in the coming hours (3—6 h). Statistical analysis (two-way ANOVA with repeated measures) showed that LPS administration increased rectal temperature after a period of ~3 h [$F(5,100) = 7.64$; $P < 0.01$; Fig. 4] when compared with vehicle/saline and vehicle/GA (1.3 μmol/site) groups. Post hoc analysis (F test for simple effect) revealed that the LPS-induced increase of rectal temperature in 3 h persisted up to 6 h [$F(3,20) = 64.4$; $P < 0.05$] when compared with vehicle/saline and vehicle/GA (1.3 μmol/site) groups.

IL-1β levels in the striatum

Since LPS injection increased the GA-induced convulsive behavior, we evaluated the effect of administrating this toxin on IL-1β levels after GA (1.3 μmol/site) injection in the striatum of rat pups. Statistical analysis showed that LPS administration increased IL-1β levels after 3[$F(3,11) = 11.49$; $P < 0.01$; Fig. 5A] and 6 h [$F(3,10) = 8.26$; $P < 0.01$; Fig. 5B] in the injected striatum. Post hoc analysis (Student—Newman—Keuls test) revealed that this increase was higher in 6[$F(3,13) = 4.58$; $P < 0.03$] than in 3 h.

Correlation analyses (Pearson's correlation coefficient) revealed a strong positive correlation between time in seizures induced by GA administration (1.3 μmol/site) and striatal IL-1β levels ($r = 0.922$; $P < 0.03$; Fig. 6) in animals injected with LPS (2 mg/kg; i.p.).

IL-1β antibody treatment

Fig. 7G—H shows that injection of the IL-1β antibody (200 ng/2 μl), 45 min before LPS prevented the cytokine-mediated reduction in the latency [187.44 ± 27.3 s; $H(3) = 17.26$; $P < 0.05$] and the total time increase of seizures [26.88 ± 1.9 s; $H(3) = 20.43$; $P < 0.05$] as compared to IL-1β denatured antibody/LPS 6 h/GA (1.3 μmol/site) group. Thus, the administration of IL-1β antibody inhibited the LPS-mediated increase in the duration of GA-induced seizures. These results are in agreement with the view that inflammatory process plays a role in GA-induced seizures (Fig. 7A—H).

Discussion

The dose—effect curve for GA revealed that the dose of 1.3 μmol/site caused seizures in rat pups, measured as decreased seizure latency and increased time in seizures. In this context, there is a significant body of evidence suggesting that GA accumulation may play a role in the convulsions and neurological impairment seen in patients with glutaric acidemia (Lima et al., 1998; Kolker et al., 2002; Fighera

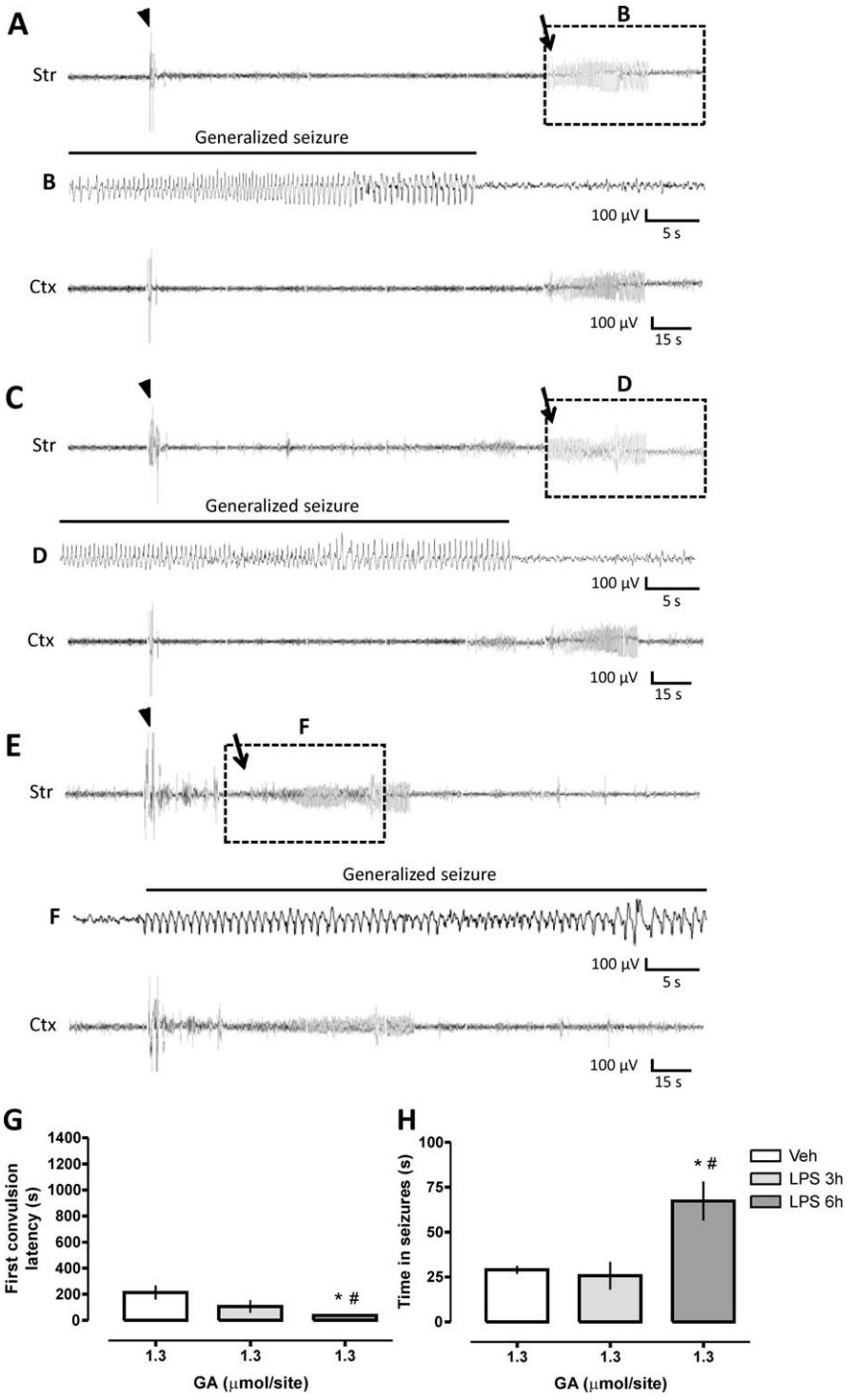

Figure 3 Representative electroencephalographic recordings: (A) intrastriatal administration of GA (1.3 μmol/site); (B) expanded waveforms from box of the EEG recording in A; (C) injection LPS (i.p.) 3 h before intrastriatal administration of GA (1.3 μmol/site); (D) expanded waveforms from box of the EEG recording in C; (E) injection LPS (i.p.) 6 h before intrastriatal administration of GA (1.3 μmol/site); (F) expanded waveforms from box of the EEG recording in E. All seizures were accompanied by pattern of continuous high-amplitude sharp spikes in low-frequency rhythmic spiking activity in ipsilateral striatum and cortex. In all traces the arrowhead indicates the GA intrastriatal administration; the arrow indicates the EEG pattern associated with generalized seizures. Str, ipsilateral striatum; Ctx, ipsilateral cortex. Calibration bars, 100 μV and 15 s. Effect of injection LPS (3 or 6 h; i.p.) before of the intrastriatal injection of GA (1.3 μmol/site) on the first convulsion latency (G) and time in seizures (H) according EEG recordings. Data are median and interquartile range for $n = 6$—8 in each group. $^{*}P < 0.05$ when compared to vehicle/GA (1.3 μmol/site) group; $^{\#}P < 0.05$ when compared to LPS 3 h/GA (1.3 μmol/site) group; Kruskal—Wallis analysis of variance.

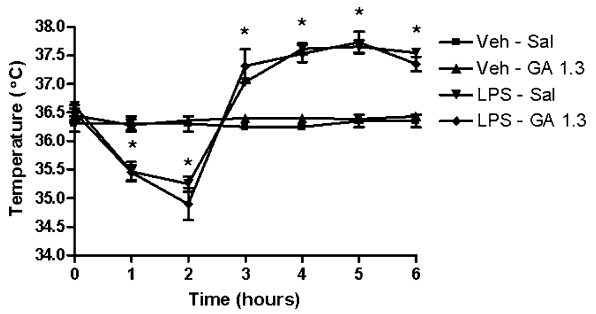

Figure 4 Effect of injection LPS (i.p.) following the intrastriatal administration of saline or GA (1.3 μmol/site) on rectal temperature from rat pups. Data are mean ± S.E.M. from absolute rectal temperatures for $n = 6$ in each group. $^{*}P < 0.01$ as compared to control group and vehicle/GA (1.3 μmol/site) group. Two-way ANOVA (Student—Newman—Keuls test).

et al., 2006). Moreover, experimental findings in vivo and in vitro suggest that energy metabolism impairment and decrease in the L-[^{3}H] glutamate uptake play an important role in the convulsive behavior elicited by GA (Porciúncula et al., 2004; Magni et al., 2009). In fact, an impairment of glutamate metabolism and homeostasis has been proposed to underlie several brain diseases, including epilepsy (Kelly and Stanley, 2001).

Since patients with GA-I are more prone to seize when presenting an infectious/inflammatory disease, we decided to investigate whether LPS administration (3 h and 6 h before GA administration) facilitated the seizures induced by this

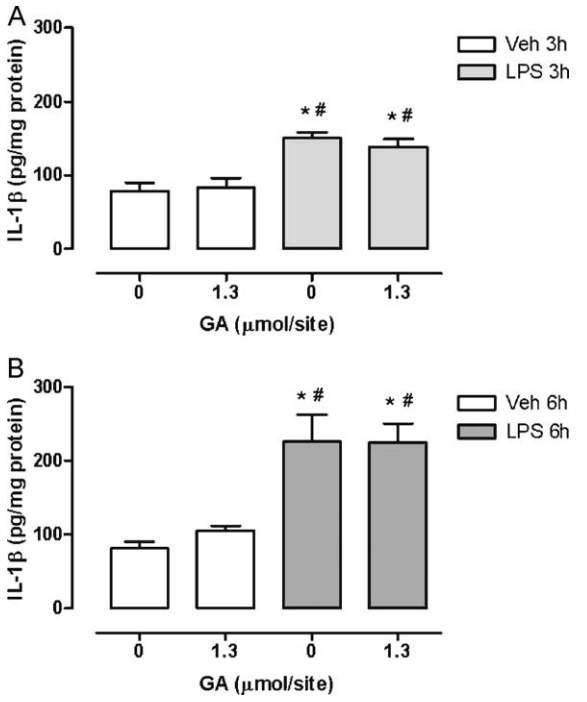

Figure 5 Effect on the IL-1β levels after administration of LPS (3 h; A and 6 h; (B) in the GA-injected striatum (1.3 μmol/site) from rat pups. Data are mean ± S.E.M from IL-1β levels for $n = 4$—5 in each group. $^{*}P < 0.01$ as compared to control group and $^{\#}P < 0.01$ as compared to vehicle/GA (1.3 μmol/site) group (A, B); Two-way ANOVA (Student—Newman—Keuls test).

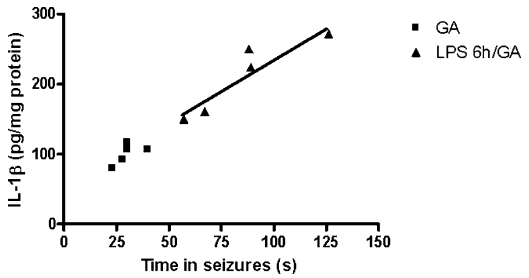

Figure 6 Increase of time in seizures induced by LPS (i.p.) 6 h before GA (1.3 μmol/site) correlates with increase IL-1β levels in the striatum (Pearson's correlation coefficient). Data are individual values for $n = 6$ in each group.

organic acid. LPS injection 6 h before intrastriatal GA injection reduced seizure latency and increased time in seizures when compared with GA alone. In this context, it has been shown that LPS administration enhances seizure susceptibility in mice injected with PTZ. Interestingly, this phenomenon is blocked by antiinflammatory drugs (Sayyah et al., 2003), suggesting the involvement of proinflammatory molecules in LPS-induced seizure susceptibility. It is worth noting that the facilitatory effect of LPS on GA-induced seizures was seen only when LPS was administered 6 h before GA, but did not at 3 h.

In addition, the LPS-induced increase of rectal temperature at 3 h was of similar magnitude of that observed at 6 h, indicating that changes in GA-induced convulsive parameters observed after LPS injection (at 6 h) is not related to an increase in body temperature.

Since LPS is a general stimulator of innate immune system to produce proinflammatory and/or cytotoxic factors as IL-1β, IL-6, TNF-α (Cohen, 2002), it has been used to induce neuroinflammation in animals (Eriksson et al., 2000; Iliev et al., 2001; Turrin et al., 2001; Turrin and Rivest, 2004; Maeda et al., 2008). Therefore, we determined the IL-1β levels in the GA-injected striatum at 3 and 6 h after LPS administration, and it observed that LPS treatment increased IL-1β levels at 3 and 6 h in a time-dependent manner.

This study does not provide a mechanism by which increased IL-1β levels facilitates GA-induced seizures in LPS-injected animals. However, our results are in agreement with previous studies that have shown that cerebral IL-1β levels are significantly higher in animals treated with LPS and kainic acid (KA) which developed seizures than in those that did not seize (Heida and Pittman, 2005). Thus, it is plausible to propose that increased neuronal excitability elicited by higher striatal IL-1β level leads to appearance of electroencephalographic and behavioral seizures induced by administration of GA and LPS. In addition, we observed a strong correlation between time in GA-induced seizures and striatal IL-1β levels in LPS-injected animals (LPS injected 6 h before GA administration). Taken together these observations, we may suggest that there should be a higher striatal IL-1β level to generate neuronal excitability and to increase the time in seizures as observed here.

In this context, it has been demonstrated that administration of IL-1β prolongs the duration of electroencephalographic and behavioral seizures induced by

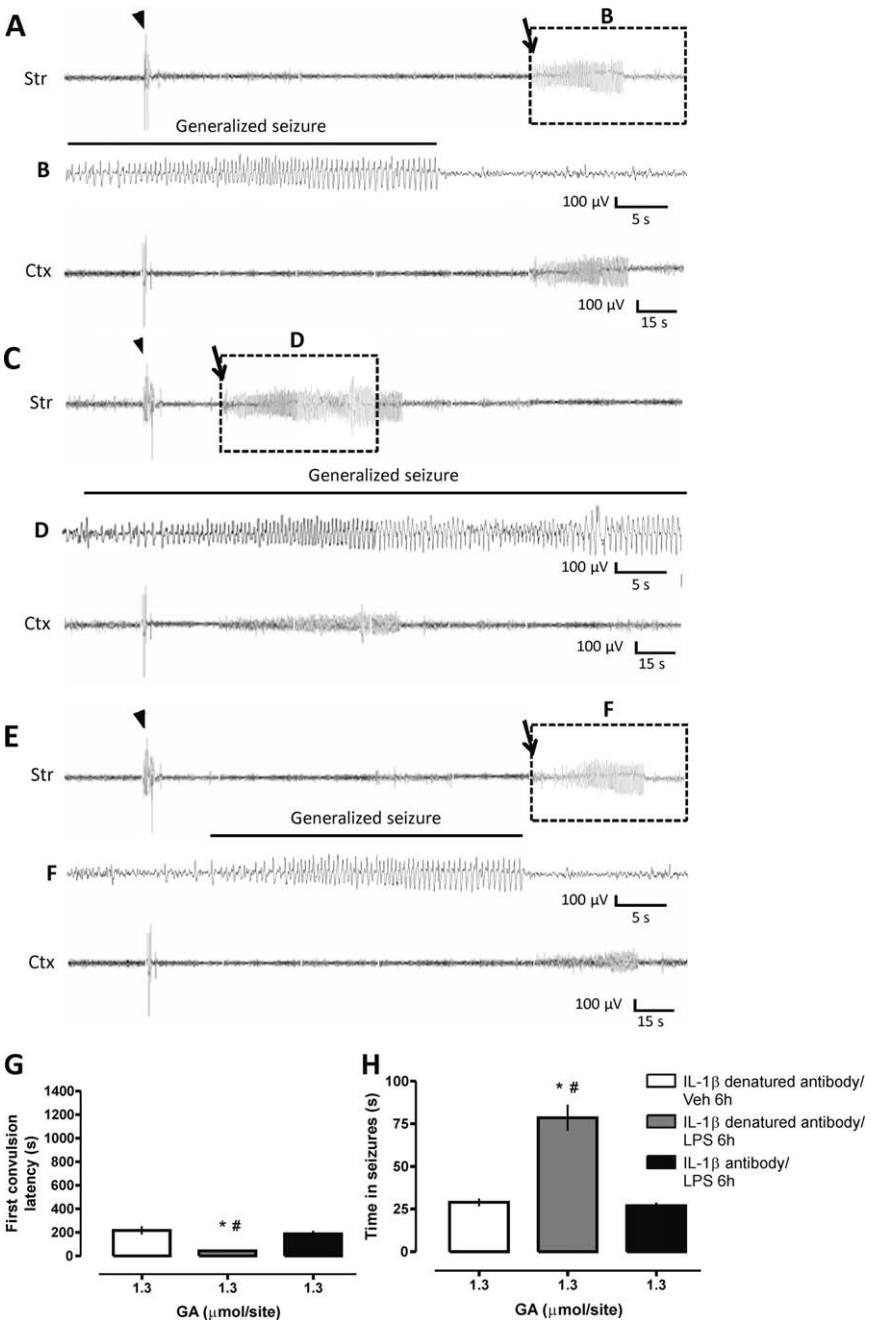

Figure 7 Representative electroencephalographic recordings: (A) intrastriatal injection IL-1β denatured antibody 45 min before vehicle (i.p.) and 6 h before intrastriatal administration of GA (1.3 μmol/site); (B) expanded waveforms from box of the EEG recording in A; (C) intrastriatal injection IL-1β denatured antibody 45 min before injection LPS (i.p.) and 6 h before intrastriatal administration of GA (1.3 μmol/site); (D) expanded waveforms from box of the EEG recording in C; (E) intrastriatal injection IL-1β antibody 45 min before LPS (i.p.) and 6 h before intrastriatal administration of GA (1.3 μmol/site), which delayed the appearance of pattern of continuous high-amplitude sharp spikes in low-frequency rhythmic spiking activity in ipsilateral striatum and cortex; (F) expanded waveforms from box of the EEG recording in E. In all traces the arrowhead indicates the GA intrastriatal administration; the arrow indicates the EEG pattern associated with generalized seizures. Str, ipsilateral striatum; Ctx, ipsilateral cortex. Calibration bars, 100 μV and 15 s. Effect of IL-1β antibody on the first convulsion latency (G) and time in seizures (H) in rat pups that received LPS 6 h before GA (1.3 μmol/ striatum). Data are median and interquartile range for $n = 6$—8 in each group. $^{*}P < 0.05$ as compared to IL-1β denatured antibody/vehicle 6 h/GA (1.3 μmol/site) group (D, E) and $^{\#}P < 0.05$ as compared to IL-1β antibody/LPS 6 h/GA (1.3 μmol/site) group (D, E); Kruskal—Wallis analysis of variance.

intracerebral application of KA in rodents (Vezzani et al., 1999). On the other hand, the intracerebral injection of IL-1-receptor antagonist (IL-1Ra) has powerful anticonvulsant effects (Vezzani et al., 2002), and transgenic mice overexpressing IL-1Ra have a reduced susceptibility to seizures (Vezzani et al., 2000), suggesting that cerebral IL-1β increase contributes to the maintenance of seizures in these models.

Current evidence indicates that cytokines, particularly IL-1β, increase neuronal excitability by activating IL-1 receptors (Vezzani et al., 1999; Bernardino et al., 2005). In this context, it has been suggested that IL-1R1-mediated modulation of glutamatergic transmission may contribute to excitotoxicity and spontaneous seizures since IL-1R1 and N-methyl-D-aspartate (NMDA) receptors colocalize in the striatum (Lawrence et al., 1998; Kwon et al., 2008) and in hippocampal pyramidal neurons (Viviani et al., 2003). In addition, the neuronal IL-1R1 stimulation induces Src kinase-mediated tyrosine phosphorylation of the NR2B subunit of the NMDA receptor. As a consequence, IL-1β facilitates NMDA receptor-mediated Ca^{2+} influx into neurons, promoting excitotoxicity (Viviani et al., 2003) and, possibly, seizure generation (Vezzani and Baram, 2007). IL-1β can also inhibit glutamate uptake in astrocytes (Hu et al., 2000) and increase its glial release possibly via TNF-α production (Bezzi et al., 2001), thus resulting in elevated extracellular glutamate levels and hyperexcitability. It is also worth pointing out that IL-1β has been shown to reduce γ-aminobutyric acid $(GABA)_A$ receptor-mediated chloride currents in hippocampal cell cultures (Wang et al., 2000), which may further add to seizure generation. Thus, since increased glutamatergic transmission and/or inhibited GABAergic function may induce seizures (Fighera et al., 2003, 2006), it is plausible that the dual actions of IL-1β on excitatory and inhibitory neurotransmission could facilitate GA-induced seizures. However, this explanation remains speculative in nature, and further studies are necessary to clarify this point.

Although the tissue concentrations of GA after intrastriatal administration are unknown, we may speculate that reduction of glutamate uptake by GA may facilitate the activation of excitatory amino acids receptors and, consequently, to generate seizures. Of note, it has been shown that glutamate transporters inhibitors do not produce seizures by themselves, but they are capable of facilitating seizures in the presence of inflammation (Liu et al., 2009). Since GA enhances synaptic turnover of glutamate and neuronal hyperexcitability (Nanitsos et al., 2004), it is plausible to propose that LPS-induced increase in IL-1β levels facilitates seizures by increasing neuronal glutamate availability. Taken together these observations, and previous reports demonstrating that GA inhibits glutamate uptake (Porciúncula et al., 2004) and that IL-1β may induce excitotoxicity by glutamatergic mechanisms (Viviani et al., 2003; Vezzani and Baram, 2007), it is conceivable that our results may be related to these findings.

We also found that anti-IL-1β antibody prevented the IL-1β-induced reduction of seizure latency and increase of time in seizures, suggesting the participation of this proinflammatory cytokine in the increased seizures susceptibility. These data suggest that IL-1β signaling present in inflammatory process contributes critically to neuronal hyperexcitability and consequently in GA-induced reduction of seizure latency and increase of time in seizures. This finding is reinforced by the evidence that impairment of the endogenous production of IL-1β by using selective blockade, or gene knockout of caspase-1, the enzyme producing the biologically active form of IL-1β, significantly reduces seizures (Ravizza et al., 2006).

In conclusion, since IL-1β can exacerbate seizures, inhibit neurogenesis and increase blood—brain barrier permeability (Quagliarello et al., 1991; Ekdahl et al., 2003), pharmacologic approaches specifically targeted to block the overproduction of IL-1β or its function(s) in glutaric acidemia may represent new nonconventional strategies for the treatment of seizure in this disorder. However, clinical studies shall be conducted in order to evaluate its clinical efficacy in glutaric acidemic patients.

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We confirm that we have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. In addition, we would like to state that all authors have seen and approved the study and that no part of the work has been published or is under consideration for publication elsewhere. Moreover, the present study was supported by government funding and has no financial or other relationship that might lead to a conflict of interest. We also would like to declare that all experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996, and that the University Ethics Committee approved the respective protocols.

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