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Exposure to prenatal stress enhances the development of seizures in young rats

Lihle Qulu · Willie M. U. Daniels · Musa V. Mabandla

Abstract A febrile seizure is a neurological disorder that occurs following an infection that results in a rapid rise in body temperature. It commonly affects 3–5% of children between the ages of 3 months and 5 years. Interleukin-1 beta IL-1β a pro-inflammatory cytokine has been suggested to play a role in the manifestation of febrile seizures. There is evidence suggesting that neurological disorders can be exacerbated in an offspring that was exposed to stress prenatally. The aim of our study was therefore to investigate whether febrile seizures are exacerbated in the offspring of rats that were prenatally stressed. The offspring of pregnant Sprague–Dawley dams were used in the study. Prenatal stress consisted of exposing the pregnant dams to 45 min of restraint, 3 times per day with 3 h intervals in-between, for 7 days starting on gestational day 14 (GND14). On postnatal day (PND) 14, the pups were injected with lipopolysaccharide (LPS, 200 μg/kg, i.p.) followed 2.5 h later by an i.p. injection of kainic acid (KA, 1.75 mg/kg). All the animals were decapitated on PND 21. Trunk blood was collected to detect plasma interleukin-1beta (IL-1β) levels in the various groups. Our data showed that i.p. injections of LPS followed by KA led to the development of seizure activity that was associated with increased plasma IL-1β

levels. Prior exposure to prenatal stress resulted in the development of advanced stages of seizure development, leading to an exaggerated seizure response. Prenatal stress alone also led to elevated plasma IL-1β levels, while previously stressed animals receiving LPS and KA yielded the highest plasma levels of IL-1β levels. Our data therefore shows that IL-1β levels may play an important role in the development of febrile seizures.

Keywords Febrile seizures · Prenatal stress · Interleukin-1beta · Kainic acid · Lipopolysaccharide

Introduction

A febrile seizure is a neurological abnormality that occurs following an infection associated with a rapid rise in body temperature (Dube et al. 2005). Africa is estimated to have the highest incidence of febrile seizures influenced by conditions such as malnutrition due to low socio-economical lifestyle (Idro et al. 2008; Diop et al. 2003). These seizures commonly affect 3–5% of children between the ages of 3 months and 5 years (Scantlebury and Heida 2010; Dube et al. 2004) and are often associated with infections of the upper respiratory tract, the middle ear (otitis media) and the gastrointestinal tract (Gulec and Noyan 2001). There are three types of febrile seizures viz: 1) a simple febrile seizure: these seizures occur during a febrile infection and have a life span of less than 15 min.; 2) a complex febrile seizure: this seizure has a life span of 15–30 min and is characterized by more than one seizure per episode of fever. Complex febrile seizures have a tendency of reoccurring; 3) Status epilepticus: here seizures occur arbitrarily in the brain during a febrile infection and have a life span greater than 30 min (Scantlebury and Heida 2010). They may reoccur within

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24 h (Heida et al. 2009; Dube et al. 2007). Simple febrile seizures are considered benign while it has been suggested that complex and status epilepticus seizures can later develop into more severe conditions such as temporal lobe epilepsy (Heida et al. 2009; Baram et al. 1997).

A seizure is commonly defined as a sudden higher than normal firing of neurons in the brain. However the cause of this sudden firing remains unclear (Vezzani et al. 2011). Studies have shown a link between febrile seizures and the activation of the immune system during a fever (Riazi et al. 2010; Dube et al. 2009; Kira et al. 2005). This activation of the immune system during a fever results in the release of pro-inflammatory cytokines that include tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β) and other cytokines such as IL-6 and IL-18 (Heida et al. 2009; Rijkers et al. 2009; Dube et al. 2007; Heida and Pittman 2005).

Prenatal stress is the exposure of an expectant mother to distress and can lead to neurological disorders in the offspring (Patin et al. 2004a,b; Berger et al. 2002). It has been suggested that these abnormalities may stem from modifications in intracellular processes within neurons that may lead to their dysfunction later in life (Mabandla and Russell 2010). Repetitive activation of the hypothalamic-pituitary-adrenal (HPA) axis during frequent bouts of stress often results in elevated concentrations of glucocorticoids in both the peripheral and central circulation (Mabandla et al. 2009a,b; Kofman 2002). Abnormally high levels of glucocorticoids have been shown to be toxic to regions of the nervous system that are easily excitable such as the pyramidal cells of the hippocampus. These regions may therefore be intimately involved in the development of seizure activity (McEwen and Magarinos 2001; Weinstock 2007).

The aim of our study was therefore to investigate whether the development of febrile seizures are exacerbated in the offspring of rats that were prenatally stressed.

Methods and materials

Animals

Sprague–Dawley rats were used in the experiments. They were obtained from the Biomedical Resource Center of the University of KwaZulu-Natal, where they were housed under standard laboratory conditions of 22°C room temperature, 70% humidity, and a 12 h light/dark cycle (lights on at 06 h00). Food and water was freely available. All experimental procedures were approved by the Animal Ethics Research Committee of the University of KwaZulu-Natal and were in accordance with the guidelines of the National Institutes of Health, USA. Ethical clearance number was 041/11.

Ten female and five male rats were used for mating. When the female rat was in pro-estrous, a male was

introduced into the cage and vaginal smears were taken the following morning. The presence of sperm in the smear indicated successful mating and was regarded as gestational day 0 (GND 0). Following a successful mating the male rat was removed from the cage.

Prenatal handling

On gestational day 14 (GND 14) the pregnant rats were divided into two groups namely a non-stressed ($n=6$) and a stressed group ($n=8$). The stressed group of rats was taken to a separate room where the animals were placed in rodent restrainers for 45 min, three times a day with 3 h intervals between successive stress periods. The stress paradigm started at 08 h00. The rats were returned to the housing room at the end of each stress period. Animals were subjected to restraint stress for 7 days until GND 20. The non-stressed rats were left undisturbed in their home cages.

Induction of seizures

Following birth, all the offspring irrespective of gender were used in subsequent experiments since hormonal changes in females were irrelevant at this early stage of the pups' lives. The pups remained with their dams until postnatal day 14 (PND14). On PND14 pups were taken from their dams and placed in new cages. The pups were taken to the experimental room one hour before the injection of lipopolysaccharide (LPS) and kainic (KA) acid. This was done to allow the animals to acclimatize to the new environment. To induce the febrile seizures, the animals received 200 µg/kg of LPS intraperitoneally (i.p.), followed 2.5 h later by an injection of kainic acid: 1.75 mg/kg, i.p. (Heida et al., 2004).

Assessment of seizure activity

One hour after the kainic acid injection, the behavior of each rat was video recorded over a 90 min period for signs of seizure activity. Seizure activity was later scored by two independent individuals. The rat's response was scored according to parameters described by others (Erakovic et al. 2001; Ojewole 2008) and was as follows: Stage 0 - no response; Stage 1 - ear and facial twitching; Stage 2 - convulsive waves throughout the body; Stage 3 - myoclonic jerks and rearing; Stage 4 - clonic convulsions with animal falling on its side; Stage 5 - repeated severe tonic-clonic convulsions or fatal convulsions.

Determination of plasma interleukin-1beta levels

On PND 21 all the animals were decapitated and trunk blood was collected for the interleukin-1beta assay. Plasma levels of Interleukin 1-beta were measured using a commercially

available ELISA kit (BioLegend, Inc, San Diego, USA). For this part of the experiment two additional control groups of rats were included in the study. The first consisted of non-stressed animals receiving saline injections (NS-Saline), while the second group was animals that were subjected to prenatal stress and injected with saline (S-Saline). There were 6 animals in each group.

Statistical analysis

The data was analyzed with the software program Graph Pad Prism (version 5). For the behavioral data qualitative statistics were used (Chi-squared test), while non-parametric tests (Kruskal-Wallis followed by Mann–Whitney test) were applied to determine the significance of differences for the interleukin-1beta data. Differences were considered significant when p-value <0.05.

Results

Assessment of seizure activity

To induce seizure activity, 14-day-old pups were injected with lipopolysaccharide (LPS) and 2.5 h later with kainic (KA) acid. The progressive development of seizures in the non-stressed (NS) group of animals subjected to this procedure i.e. NS,LPS,KA was the following: one animal out of six exhibited features of stage one; all six animals displayed signs of stages two and three; and only two animals in the group went on to develop features of stage four. No animals showed seizure activity associated with stage five (Fig. 1).

The development of seizure activity in the stressed (S) group of animals was significantly different to that of the non-stressed group (Fig. 2). Stressed animals injected with LPS and KA showed the following progressive seizure development pattern: none of the eight animals showed

signs of stage one; six out of eight developed stage two symptoms; one animal displayed features of stage three; six animals exhibited characteristics of stage four; and all eight animals went on to develop stage five features (Fig. 2).

Plasma interleukin- 1beta (IL-1β) levels

Comparison of the interleukin-1beta (IL-1β) concentrations between the various groups of animals showed significant differences (Fig. 3, $p<0.05$ in all comparisons). The non-stressed LPS/KA-treated (NS,LPS,KA) group of animals had a significantly higher concentration of IL-1β compared to the non-stressed saline-treated (NS-Saline) group. There was also a significant difference in IL-1β levels of NS-Saline animals vs. stressed saline-treated (S-Saline) animals, with the NS-Saline group having lower concentrations of IL-1β than the S-Saline group. Comparison between the two groups of stressed animals showed that animals that received LPS, KA injections (S,LPS,KA) had significantly higher levels of IL-1β than the S-Saline control group.

Discussion

In this study we investigated the effects of prenatal stress on febrile seizures and in particular determined whether the development of febrile seizures is exacerbated in the offspring of rats that were prenatally stressed. We exposed pregnant dams to restraint stress using rodent restrainers, similar to previous reports (Fumagalli et al. 2007). Pregnant rats were exposed to stress from gestational day 14, since this age is considered the initiation of gross neural structural differentiation in the fetal brain (Patin et al. 2004a,b; Berger et al. 2002). Subsequently febrile seizures were induced by the combined injection of lipopolysaccharide (LPS) and kainic acid (KA) on postnatal 14, an age equivalent to human infants between 1 and 2 years (Heida et al. 2004).
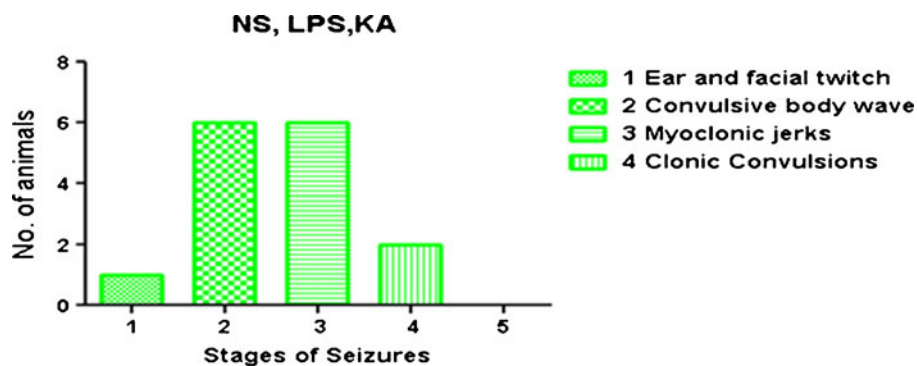

Fig. 1 A graph showing the presence of seizure activity in non-stressed (NS) animals injected with lipopolysaccharide (LPS: 200 μg/kg, i.p.) followed 2.5 h later by kainic acid (KA: 1.75 mg/kg, i.p.) during the different stages of seizure development. Results show the number of animals that displayed the particular features of a specific stage during the development of seizures. There was a total of 6 animals in this group
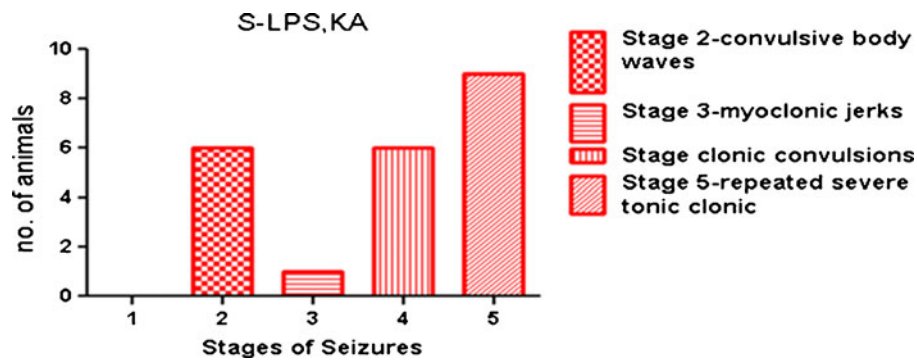
Fig. 2 A graph showing the presence of seizure activity in prenatally (S) animals injected with lipopolysaccharide (LPS: 200 μg/kg, i.p.) followed 2.5 h later by kainic acid (KA: 1.75 mg/kg, i.p.) during the different stages of seizure development. Prenatal stress consisted of exposing pregnant dams to 45 min of restraint stress, 3 times per day with 3 h intervals, for 7 days, between gestational day 14–20. Results show the number of animals that displayed the particular features of a specific stage during the development of seizures. There was a total of 8 animals in this group

Our data showed that treating pups with LPS and KA resulted in the development of seizure activity that ranged from ear and facial twitches (Stage 1) to some animals showing clonic convulsions with animals falling on its side (Stage 4). The proposed mechanism of action by which this strategy induces seizures involves LPS triggering the activation of the enzyme cyclooxygenase-2 (COX-2) which catalyzes the transformation of arachidonic acid into prostaglandin E2 (PGE2) (Heida et al. 2009). Prostaglandin E2 targets the hypothalamus where it raises the body's "set point" thus increasing the core body temperature. This increase in body temperature in response to LPS triggers the release of cytokines such as IL-1β (Heida et al. 2009). The pro-inflammatory response that triggers the release of IL-1β also initiates the synthesis and release of IL-1 receptor antagonist (IL-1ra), which possesses anti-convulsing effects (Heida and Pittman 2005). It competitively binds to IL-1R1 thus blocking IL-1β effects (Rijkers et al. 2009; Heida and Pittman 2005). However this competitive binding between IL-1ra and IL-1β favors IL-1β and results in an imbalance between IL-1β and IL-1ra, which in turn triggers the onset of febrile seizure (Rijkers et al. 2009; Heida and Pittman 2005). It has been proposed that IL-1 β acts by altering the function of glutamate in the central nervous system (Galic et al. 2008; Spencer et al. 2005).

Glutamate is an excitatory neurotransmitter that exerts its postsynaptic effects through 3 classes of ionotropic receptor channels viz: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate (KAR) and N-methyl-D-aspartate (NMDA) receptors. Kainic acid receptor subtypes (KAR 1 and KAR 2) are largely distributed in the CA2-3 and dentate gyrus regions of the hippocampus (Bunch and
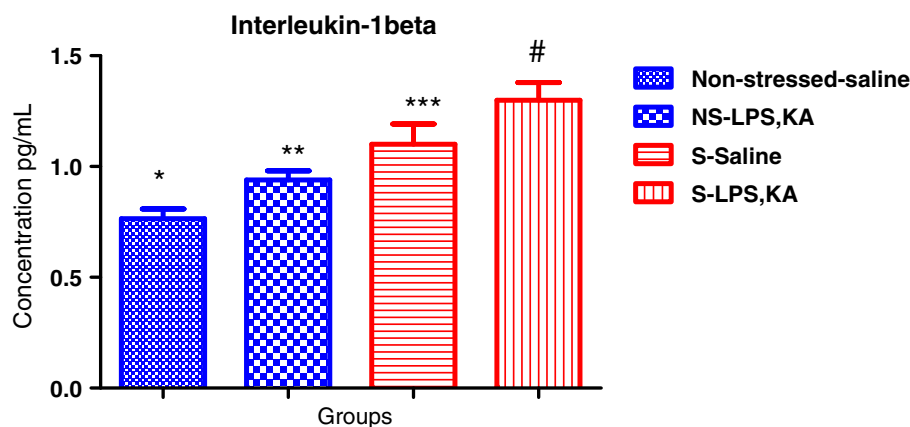

Fig. 3 A graph showing the plasma concentration of IL-1β in the different groups of animals. Two additional saline-treated control groups were included in this part of the study. Data is presented as the mean±SEM of 6 samples. *$p<0.05$: Non-stressed, saline treated group vs Non-stressed, lipolysaccharide, kainic acid treated group, Mann–Whitney test. **$p<0.05$: Non-stressed, saline treated group vs Stressed, saline treated group, Mann–Whitney test. ***$p<0.05$: Stressed, saline treated group vs Stressed, lipolysaccharide, kainic acid treated group, Mann–Whitney test. #$p<0.05$: Non-stressed, lipolysaccharide, kainic acid treated group vs Stressed, lipolysaccharide, kainic acid treated group, Mann–Whitney test

Larsen 2009; Lujan et al. 2005). The kainic acid receptors have been shown to have a high affinity for glutamate in comparison to the AMPA receptors (Bunch and Larsen 2009). Kainic acid binds to the KAR that evokes seizures due to an increase in the excitation of the glutamate receptors in the hippocampus (Bunch and Larsen 2009).

In our study we specifically used a concentration of kainic acid that was unable to initiate seizure activity on its own. Therefore the development of seizures in our experiments was dependent on the animals being pretreated with LPS. In this sense we were comfortable that our animal model was a reasonable reflection of febrile seizures in humans. In addition our observations were in agreement with previous studies showing postnatal exposure to LPS leading to a higher seizure susceptibility to convulsants such as KA (Galic et al. 2008).

Prior exposure to prenatal stress, followed by an immune challenge of LPS resulted in more complex and intense seizure activity. Our results showed elevated plasma levels of IL-1β in our stressed saline-treated group compared to the non-stressed saline-treated group. It is therefore possible that the exaggerated seizure activity in the stressed group may have resulted from greater increased levels of IL-1 β. As mentioned earlier IL-1β is one of the prime pro-inflammatory cytokines that has been implicated in the manifestation of fever-related seizures (Vezzani and Baram 2007).

Alternative mechanisms by which prenatal stress could have led to the exaggeration of seizure activity include alterations in the activity of the hypothalamus-pituitary-adrenal axis of the offspring. Prenatal stress has been reported to reduce systemic blood flow that may induce conditions of oxidative stress. In turn the stressful environment may indirectly lead to the release of cytokines in placental tissue, thereby contributing to neurological maldevelopment (Charila et al. 2010). Furthermore there is good evidence showing IL-1β activating the HPA axis and triggering the release of glucocorticoids (Besedovsky et al. 1986; Wang and Dunn 1999; Burckingham et al. 1994). For instance Wang and Dunn (1999) showed that treating mice with IL-1β resulted in increased plasma corticosterone levels, a finding supported by a similar study by Burckingham et al. (1994).

In conclusion our data shows that an immune challenge followed by a sub-lethal neurological insult, can lead to the development of seizure activity. The development of these seizures may be due to increased release of IL-1β. Our findings also suggest that prenatal exposure to stress can result in more intense seizure development, a process mediated by enhanced IL-1β release and/or stress-induced increases in circulating glucocorticoids. Our study therefore may provide some insight into the high prevalence of febrile seizures in children of the African continent where stress in utero is common.

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