Published in final edited form as:

Epilepsia. 2012 March ; 53(3): e46–e49. doi:10.1111/j.1528-1167.2011.03357.x.

Prenatal stress promotes development of spasms in infant rats

Mi-Sun Yum^{*,1}, Tamar Chachua^{†,1}, Jana Velíšková^{†,‡}, and Libor Velíšek^{†,§}

^{*}Department of Pediatrics, Asan Medical Center, Seoul, Republic of Korea

[†]Department of Cell Biology & Anatomy, New York Medical College, Valhalla, New York, U.S.A

[‡]Department of Obstetrics & Gynecology, New York Medical College, Valhalla, New York, U.S.A

[§]Department of Pediatrics, New York Medical College, Valhalla, New York, U.S.A

Summary

We have developed a new model of cryptogenic infantile spasms with prenatal betamethasone brain priming to increase susceptibility to development-specific spasms triggered by *N*-methyl-D-aspartate (NMDA). A recent clinical study linked severe prenatal stress to increased risk for development of infantile spasms. Here, we determined whether prenatal restraint stress (2×45 min) in rats on gestational day 15 would increase susceptibility to develop spasms on postnatal day 15. Prenatal stress significantly accelerated onset and increased number of NMDA-triggered spasms compared to handled controls. A single adrenocorticotrophic hormone (ACTH or corticotropin) dose delivered acutely had no effects, whereas long-term (3 day) ACTH pretreatment significantly increased latency to onset and decreased number of spasms (an effect similar to that in the human condition). Our data support the notion that extra care should be provided during pregnancy to minimize stress.

Keywords

Infantile spasms; Model; ACTH; Prenatal; Stress

We have developed a novel rat model of cryptogenic infantile spasms consisting of prenatal priming with beta-methasone and postnatal trigger of spasms with *N*-methyl-D-aspartate (NMDA) (Velíšek et al., 2007). Prenatal priming impairs homeostasis in the hypothalamo-pituitary-adrenal axis similar to the human condition (Nalin et al., 1985; Baram et al., 1992; Owen & Matthews, 2003) and postnatal NMDA triggers bouts of flexion spasms with accelerated incidence compared to prenatally nonprimed pups. These spasms occur at the age developmentally relevant for infantile spasms; they have similar semiology with ictal electroencephalography (EEG) electrodecrement and behavioral deterioration, and respond to corticotropin (adrenocorticotrophic hormone, ACTH) treatment (Chachua et al., 2011).

© 2011 International League Against Epilepsy

Address correspondence to Dr. Libor Velíšek, Department of Cell Biology & Anatomy, New York Medical College, 40 Sunshine Cottage Rd, BSB-A20, Valhalla, NY 10595, U.S.A. Libor_Velisek@nymc.edu.

¹These two authors contributed equally.

Formerly: The Saul R. Korey Department of Neurology (Mi-Sun Yum, Tamar Chachua, Jana Velíšková, Libor Velíšek) and Dominick P. Purpura Department of Neuroscience (Jana Velíšková, Libor, Velíšek) Albert Einstein College of Medicine, Bronx, NY, U.S.A.

Disclosure

None of the authors has any conflict of interest to disclose. 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.

Stress delivered to pregnant mothers also elevates prenatal corticosteroids (Weinstock, 2008). Therefore, we decided to use prenatal stress at the same time points of pregnancy as betamethasone injections to investigate whether stress may contribute to increased susceptibility to spasms. In addition a recent report associated severe prenatal stress with increased risk for development of infantile spasms (Shang et al., 2010).

Methods

Experiments have been approved by the institutional animal care and use committees of the Albert Einstein College of Medicine and New York Medical College and conform to the *NIH Guide for the Care and Use of Laboratory Animals*. Timed pregnant Sprague-Dawley rats purchased from Taconic Farms (Germantown, NY, U.S.A.) were housed individually in the animal facility with lights on at 07:00 and off at 19:00 hours with free access to food and water.

On gestational day 15 (G15), pregnant rats were placed in a commercial restrainer for 45 min at 08:00 and 18:00 hours under bright light. The timing reflects our prenatal betamethasone priming (Chachua et al., 2011). We chose restraint stress because of simplicity and relatively good reproducibility. Although restraint stress has a potential for induction of mechanical trauma in fetus, such effects have not been reported previously in studies using restraint stress three times daily for 7 days (Vallee et al., 1996; Edwards et al., 2002). Control pregnant rats were handled: they were picked up, held for 10 s, and returned back to the home cage. No more than two male and two female pups from prenatally stress-primed or handled litters were used per subgroup to avoid systematic error ("litter effect"). On postnatal day 15 (P15), pups were placed in observation cages on a heated pad. We tested the following: (1) the susceptibility to develop spasms after NMDA administration (15 mg/kg, i.p.); (2) in different prenatally stressed or handled pups the effects of ACTH (full 39 aminoacid rat ACTH; GenScript, Piscataway, NJ, U.S.A.) (a) in the acute pretreatment with 0.1 mg/kg, i.p. [effective in our original model (Velíšek et al., 2007)] or 0.3 mg/kg, s.c. 1 h before NMDA and (2) in long-term pretreatment for 3 days (three doses of 0.3 mg/kg ACTH s.c. daily on P12–14 at 07:00, 14:00, and 21:00 hours). Controls received saline injections. We determined latency to onset of spasm and the total number of spasms per 75-min observation period (the spasms terminate within 1 h after the NMDA injection).

Initial statistical evaluation never found sex differences (p-value always >0.22); therefore, male and female animals were combined. Analysis of variance (ANOVA) with post hoc Fisher protected least square degree test was used with level of significance set to $p < 0.05$ and adjusted for multiple comparisons.

Results

Two episodes of prenatal restraint stress did not have any significant effect on the number of pups in the litter or their body weight on P15 prior to any additional treatments (n-handled = 24; n-stressed = 22; $F_{1,44} = 0.702$; $p = 0.4067$; Fig. 1A).

The sequence of NMDA-induced behavioral events was similar in all groups; only the timing and number of events were different. After the injection, the rats rested quietly; they even fell asleep for about 10 min. After this period they became hyperactive, were running around, and developed tail twisting. Next the rats fell on one side and developed partial flexion without a contribution of head/neck (Fig. 1B). This stage evolved into full spasms (hyperflexion of head/neck, spine, and tail) with multiple repetitions (Chachua et al., 2011).

There was no significant difference in the latency to onset of tail twisting ($F_{1,20} = 1.564$; $p = 0.2255$; Fig. 1C) between offspring of prenatally handled and stressed mothers. However, prenatally stressed rats developed the spasms significantly earlier compared to controls ($F_{1,20} = 8.945$; $p = 0.0072$; Fig. 1D). The number of spasms was also significantly increased in prenatally stressed animals compared to handled controls ($F_{1,19} = 7.638$; $p = 0.0124$; Fig. 1E). When we calculated frequency of spasms normalized per 1 h period, the contrast between the groups was even increased ($F_{1,19} = 21.278$; $p = 0.0002$, Fig. 1F).

Acute ACTH pretreatment

There was no main effect of ACTH (0.1 mg/kg, i.p., 1 h prior to NMDA) on either latency to onset of spasms (two-way ANOVA; $F_{1,18} = 1.013$; $p = 0.3275$; Fig. 2A), or on the number of spasms ($F_{1,18} = 0.028$; $p = 0.8678$; Fig. 2B). Conversely, regardless of the postnatal treatment (ACTH or saline), prenatal stress exposure was consistently associated with earlier onset of spasms ($F_{1,18} = 9.062$; $p = 0.0075$; Fig. 2A) as well as with a higher number of spasms ($F_{1,18} = 7.457$; $p = 0.0137$; Fig. 2B) compared to controls. In different groups of prenatally stressed rats, 0.3 mg/kg of ACTH, s.c. (used in chronic pretreatment paradigm; $n = 6$) did not have any effect on either latency to onset of spasms ($F_{1,9} = 0.709$; $p = 0.422$; Fig. 2C) or number of spasms ($F_{1,9} = 0.221$; $p = 0.650$; Fig. 2D) compared to controls ($n = 5$).

Chronic ACTH pretreatment

In this experiment, we used only the rats prenatally exposed to stress. Chronic ACTH significantly increased latency to onset of spasms ($n = 8$; $F_{1,12} = 21.656$; $p = 0.0006$; Fig. 2E) compared to chronic administration of saline ($n = 6$). Similarly, chronic ACTH significantly suppressed number of spasms occurring per observation period ($F_{1,12} = 13.680$; $p = 0.0030$; Fig. 2F). In addition, we found that there was no difference in body weight gain between pups randomly assigned to chronic ACTH or vehicle groups from P1 to P12 ($F_{1,12} = 0.016$; $p = 0.9006$; Fig. 2G). However, chronic ACTH pretreatment from P12 to 14 was associated with a significant lag in body weight gain ($F_{1,12} = 13.166$; $p = 0.0035$; Fig. 2H).

Discussion

Our study demonstrates that the NMDA-triggered spasms in immature rats are accelerated and increased in number and frequency after prenatal exposure to restraint stress compared to handling. Although the absolute numbers are different from our original study [Velíšek et al., 2007], probably because of differences in animals, drug batch, and so on], the direction of the differences is the same. This finding is consistent with a study showing that repeated early and mid/late gestational restraint stress (although the 45-min stress was applied three times a day for 7–8 days) decreases threshold for amygdala kindling and increases kindling rate in immature, P14 rats (Edwards et al., 2002), that is, it has proconvulsant effects. Prenatal betamethasone exposure used in our previous study resulted in similar effects as prenatal stress on the latency of NMDA-induced spasms as well as on the number of spasms (Chachua et al., 2011). Relevance of this study for infantile spasms is supported by a recent report showing association of severe stress during pregnancy with increased risk to develop infantile spasms (Shang et al., 2010). Combination of these results indicates that pregnant mothers should be relieved from stress as much as possible to decrease the risk of negative outcomes in the offspring.

Neither prenatal exposure to stress nor betamethasone (Velíšek et al., 2007) was associated with development of spontaneous spasms; an additional provoking factor (NMDA injection) was used. In humans, infantile spasms also do not follow each severe gestational stress,

there is only an increased risk for occurrence of spasms (Shang et al., 2010). This indicates that additional perinatal or post-natal factors are required for expression of infantile spasms.

Acute administration of ACTH (0.1 or 0.3 mg/kg) was ineffective against the spasms in prenatally stressed pups, although previously after prenatal betamethasone exposure, the 0.1 mg/kg dose of ACTH significantly delayed the onset of spasms (Velíšek et al., 2007). This differential outcome suggests either different mechanisms or different severity of these prenatal exposures. However, chronic pretreatment with ACTH was definitely effective, suggesting that this model, similar to the human condition of infantile spasms (Baram et al., 1996), may require long-term ACTH treatment.

We have not seen any effects of prenatal stress on postnatal body weight, although decreases in body weight have been described after significant prenatal restraint stress (three times daily) between G14 and G21 (Vallee et al., 1996). On the other hand, exposure to one random stressor daily between G13 and G20 had no effect on body weight (Zohar & Weinstock, 2011). We have shown that chronic ACTH treatment is associated with decreases in body weight. This effect may be due to ACTH effects on food intake or growth, which is consistent with the effects of melanocortin-4 receptors agonists in obesity treatments (Emmerson et al., 2007).

Our study indicates that prenatal stress may significantly enhance susceptibility to develop triggered spasms in infant rats; this finding is similar to increased risk for development of infantile spasms in children of mothers with severe gestational stress.

Acknowledgments

This work was supported by National Institutes of Health grants NS059504, NS056093, and NS072966, and by March of Dimes Foundation grant #6-FY08-214.

References

- Baram TZ, Mitchell WG, Snead OC, Horton EJ, Saito M. Brain-adrenal axis hormones are altered in the CSF of infants with massive infantile spasms. *Neurology*. 1992; 42:1171–1175. [PubMed: 1318521]
- Baram TZ, Mitchell WG, Tournay A, Snead OC, Hanson RA, Horton EJ. High-dose corticotropin (ACTH) versus prednisone for infantile spasms: a prospective, randomized, blinded study. *Pediatrics*. 1996; 97:375–379. [PubMed: 8604274]
- Chachua T, Yum M-S, Velíšková J, Velíšek L. Validation of the rat model of cryptogenic infantile spasms. *Epilepsia*. 2011; 52:1666–1677. [PubMed: 21854372]
- Edwards HE, Dortok D, Tam J, Won D, Burnham WM. Prenatal stress alters seizure thresholds and the development of kindled seizures in infant and adult rats. *Horm Behav*. 2002; 42:437–447. [PubMed: 12488110]
- Emmerson PJ, Fisher MJ, Yan LZ, Mayer JP. Melanocortin-4 receptor agonists for the treatment of obesity. *Curr Top Med Chem*. 2007; 7:1121–1130. [PubMed: 17584132]
- Nalin A, Facchinetti F, Galli V, Petraglia F, Storchi R, Genazzani AR. Reduced ACTH content in cerebrospinal fluid of children affected by cryptogenic infantile spasms with hypsarrhythmia. *Epilepsia*. 1985; 26:446–449. [PubMed: 2995025]
- Owen D, Matthews SG. Glucocorticoids and sex-dependent development of brain glucocorticoid and mineralocorticoid receptors. *Endocrinology*. 2003; 144:2775–2784. [PubMed: 12810530]
- Shang NX, Zou LP, Zhao JB, Zhang F, Li H. Association between prenatal stress and infantile spasms: a case-control study in China. *Pediatr Neurol*. 2010; 42:181–186. [PubMed: 20159427]
- Vallee M, Mayo W, Maccari S, Le Moal M, Simon H. Long-term effects of prenatal stress and handling on metabolic parameters: relationship to corticosterone secretion response. *Brain Res*. 1996; 712:287–292. [PubMed: 8814904]

- Velíšek L, Jehle K, Asche S, Velíšková J. Model of infantile spasms induced by *N*-methyl-D-aspartic acid in prenatally impaired brain. *Ann Neurol*. 2007; 61:109–119. [PubMed: 17315208]
- Weinstock M. The long-term behavioural consequences of prenatal stress. *Neurosci Biobehav Rev*. 2008; 32:1073–1086. [PubMed: 18423592]
- Zohar I, Weinstock M. Differential effect of prenatal stress on the expression of corticotrophin-releasing hormone and its receptors in the hypothalamus and amygdala in male and female rats. *J Neuroendocrinol*. 2011; 23:320–328. [PubMed: 21306450]

Figure 1.

Two episodes of prenatal restraint stress do not affect postnatal weight, but accelerate the onset of NMDA-induced spasms and increase their number in infant rats. **(A)** Prenatal exposure to two, 45-min episodes of restraint stress on gestational day 15 (G15) did not affect body weight in pups ($n = 22$) on postnatal day 15 (P15) compared to pups ($n = 24$) whose mothers were only handled on the same day of pregnancy [$p = 0.4067$; mean $\pm$ standard error of the mean (SEM)]. **(B)** Spasms consisting of hyperflexion of head and neck, spine, as well as of tail were triggered by NMDA (15 mg/kg, i.p., in saline) in P15 rats, whose mothers were exposed to restraint stress or handled on G15. **(C)** There was no difference in latency to onset of tail twisting (the initial NMDA-induced symptom) between P15 rats prenatally exposed to stress ($n = 10$) and controls ($n = 12$, $p = 0.2255$; mean $\pm$ SEM) **(D)** The spasms occurred significantly earlier in P15 rats exposed prenatally to stress compared to controls (* $p = 0.0072$; mean $\pm$ SEM). **(E)** The number of spasms was significantly increased in P15 rats prenatally exposed to stress compared to handled controls (* $p = 0.0124$; mean $\pm$ SEM). **(F)** If the number of spasms was recalculated as frequency (time unit = 1 h), there was significantly higher frequency of spasms in P15 rats after prenatal exposure to stress compared to controls (* $p = 0.0002$; mean $\pm$ SEM).

Epilepsia ©ILAE

Figure 2.

Prenatal restraint stress reveals antispasm effects of chronic, but not acute pretreatment with ACTH (mean $\pm$ SEM). (A) Acute pre-treatment with ACTH (0.1 mg/kg, i.p., 1 h prior to NMDA challenge) did not affect latency to onset of spasms irrespective of whether the rats were prenatally exposed to stress (n-saline = 6; n-ACTH = 4) or handled controls (n-saline = 6; n-ACTH = 6). Note that the difference between prenatally stressed and handled pups was confirmed (* p = 0.0075). There was no interaction between prenatal and postnatal factors. (B) There was no effect of acute pretreatment with 0.1 mg/kg ACTH, i.p., on the number of spasms. Yet, the previously demonstrated difference between prenatal stressed and handled groups reappeared (* p = 0.0137) without an interaction between prenatal and postnatal factors. (C) Acute pretreatment with 0.3 mg/kg ACTH, s.c. (n = 6) in prenatally stressed rats 1 h prior to NMDA challenge had no significant effects on the latency to onset of spasms compared to saline pretreatment (n = 5; p = 0.422), although this ACTH dose was effective in the chronic pretreatment paradigm (see E and F). (D) After prenatal stress, there was no effect of ACTH pretreatment with 0.3 mg/kg dose on the number of spasms compared to saline-injected rats (p = 0.650). (E) Chronic pretreatment with ACTH (3 days, three doses of 0.3 mg/kg s.c. per day) on P12–P14 (n = 8) significantly delayed onset of spasms compared to controls injected with vehicle at the same times (n = 6; * p = 0.0006) in prenatally stressed rats. (F) Similarly, chronic ACTH pretreatment significantly decreased the number of spasms compared to vehicle injections (* p = 0.003) in prenatally stressed rats. (G) Infant, prenatally stressed, rats randomized on P12 to either ACTH or vehicle groups gained body weight equally between P1 and P12 (p = 0.9006). (H) However, once the chronic ACTH treatment had been initiated on P12 in prenatally stressed rats, the ACTH-treated rats lagged in their body weight gains between P12 and P15 compared to vehicle-injected controls (* p = 0.0035).

Epilepsia ©ILAE