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Estrogen differentially alters NMDA- and kainate-induced seizures in prenatally morphine- and saline-exposed adult female rats

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Abstract

The purpose of the present study was to investigate the effects of prenatal exposure to morphine on seizure susceptibility in adult female rats. Adult female rats, exposed to saline or morphine on prenatal days 11–18, were ovariectomized (OVX) and some were injected 48 h prior to seizure testing with estradiol benzoate (EB). To assess the latency to onset of stereotypy and seizures, females received systemic injections of *N*-methyl-D-aspartate (NMDA; 150, 175, 200 mg/kg) or kainic acid (KA; 10 or 15 mg/kg). Prenatal morphine exposure increased the latency to onset of wet-dog-shakes (WDS) in both OVX and OVX, EB-injected females after the higher dose of KA. However, prenatal morphine exposure increased the latency to onset of stereotypy only in OVX, EB-injected females after the highest dose of NMDA. Prenatal morphine exposure also increased the latency to onset of seizures after the lower dose of KA, but did not change the latency to onset of NMDA-induced seizures. Additionally, an EB injection increased the latency to onset of seizures in both saline- and morphine-exposed females after the lowest dose of NMDA, but decreased the latency to onset of seizures after the lower dose of KA. Thus, the present study demonstrates that prenatal morphine exposure has different effects on the estrogen regulation of the onset of seizures and stereotypy induced by NMDA or KA in adult, OVX female rats.

Keywords: NMDA; Kainic acid; Prenatal morphine; Estrogen

1. Introduction

Opiates such as morphine influence seizure susceptibility and sensitivity to convulsant drugs [1–3,16,20]. Some studies demonstrate that acute morphine administration alters *N*-methyl-D-aspartate (NMDA)- and kainic acid (KA)-induced seizures and that the effect is dependent on the dose of morphine [1,30]. While low doses of morphine inhibit, high doses enhance NMDA- and KA-induced seizures [1,30]. Additionally, morphine withdrawal stimulates NMDA- as well as KA-induced seizures [8,16].

Our previous work showed that prenatal morphine exposure alters postnatal susceptibility to NMDA- and KA-induced seizures in young adult male rats [15]. The major effect of morphine was on stereotypy in both NMDA- and KA-induced seizures. While prenatal morphine exposure shortened the latency to onset of stereotypy in KA-induced seizures, the latency to onset of NMDA-induced stereotypy was dose dependent in both saline- and morphine-exposed males [15]. There was a decrease in the latency to onset of NMDA-induced stereotypy after the dose of 175 mg/kg and an increase after the dose of 200 mg/kg of NMDA [15]. Moreover, we have additional data demonstrating that prenatal morphine exposure alters susceptibility to seizures in a sex- and gonadal hormone-dependent manner [23–25].

Effects of gonadal hormones on seizure susceptibility has been shown in adult ovariectomized (OVX) female rats in several studies [4,5,7,9,10]. While estrogen, in OVX female rats, increases [5,27], progesterone in OVX female rats decreases seizure susceptibility [5]. It is also suggested that susceptibility to seizures varies at times of hormonal changes such as throughout the menstrual cycle, and during puberty and menopause [12,13]. However, the mechanism(s) of gonadal hormone actions on seizure susceptibility is not yet established.

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Based on our previous work investigating the effects of prenatal morphine exposure on NMDA and KA seizures in adult male rats, and studies showing effects of estrogen on seizure susceptibility, the present study tests two hypotheses: (1) prenatal morphine exposure increases NMDA- and KA-induced seizure susceptibility in adult female rats and (2) an estrogen treatment 48 h prior to NMDA- or KA-induced seizures increases seizure susceptibility in adult, OVX female rats. The following parameters were measured after NMDA and KA administration: (1) seizure susceptibility by the latency to onset of seizures and incidence of seizures and (2) latency to onset of stereotypy and incidence of stereotypy. NMDA-induced stereotypy is demonstrated by enhanced rearing and sigmoidal tail movement, and KA-induced stereotypy is demonstrated by wet-dog-shakes (WDS). These measures were considered important because our previous work showed differential effects of prenatal morphine exposure on seizures and stereotypy after NMDA or KA administration in adult male rats [15].

2. Methods

Eight-day pregnant Sprague–Dawley rats were purchased from Taconic Farms (Germantown, NY). Upon arrival, pregnant dams were weighed, housed individually in maternity cages, and maintained in a temperature controlled colony room with free access to food and water on a reversed 14-h (light):10-h (dark) cycle with lights off at 1100 h. Pregnant rats were randomly assigned to an experimental morphine- or a control saline-treated group. Morphine or 0.9% physiological saline injections were administered subcutaneously twice daily (0800 and 2000 h) on days 11–18 of gestation as described in the original work of Vathy et al. [18]. The dose of the first three morphine injections was 5 mg/kg, and the remaining injections were 10 mg/kg.

The day of birth was designated as postnatal day (PND) 0. On PND 1, morphine-exposed pups were injected intradermally with black India ink in one foot pad for identification. At the same time pups were weighed, sexed, and cross-fostered. Each mother raised half of her own and half of the adopted pups from the opposite treatment [18]. Whenever possible, equal numbers of males and females made up each litter. Litters were reduced to 10 pups. Pups were weaned on PND 25, ear-punched for identification, and housed individually. All adult female rats were OVX on PND 55, and a week later they were tested for seizures. Four groups of females were tested for NMDA- and KA-induced seizures: saline- and morphine-exposed, OVX, and saline- and morphine-exposed, OVX, estradiol benzoate (EB)-treated female rats. EB (3 μg/0.1 ml dissolved in peanut oil) was injected subcutaneously 48 h prior to seizure testing. Each OVX female received 3 μg of EB. The dose of EB was based on our previous work that assessed sexual behavior [19], such that this dose was sufficient to induce lordosis behavior in most OVX females. Only one animal from each litter was used in each condition to avoid litter effects. Each testing group contains 10 females.

Doses of NMDA and KA were based on our previous studies conducted in adult male rats [15]. Three doses (150, 175, or 200 mg/kg) of NMDA dissolved in distilled water, were administered intraperitoneally (ip). After NMDA administration, females were observed for 1 h. The latency to onset of stereotypy (enhanced rearing and sigmoidal tail movements) and clonic–tonic seizures were recorded. Different groups of female rats were injected with 10 or 15 mg/kg ip of KA, dissolved in 0.01 M phosphate-buffered saline, pH 7.4. After KA administration, females were observed for 2 h. The latency to onset of WDS and clonic seizures were recorded.

A two-way ANOVA (prenatal drug exposure × adult hormone treatment) with Student–Newman–Keuls post-hoc test was used for statistical analysis of latencies to onset of stereotypy and seizures. To investigate the effects of prenatal morphine exposure on seizure susceptibility as it is regulated by estrogen in adult, OVX female rats, each dose of NMDA or KA was analyzed separately. Additionally, Fishers' test was used for comparison of incidences of stereotypy and seizures between saline- and morphine-exposed animals, separately for each dose and hormone treatment. Differences were considered significant if $P < .05$.

3. Results

3.1. NMDA-induced seizures

In NMDA-induced stereotypy, there was a main effect of hormone [$f(1,24) = 4.82$, $P < .05$] and an interaction between prenatal drug exposure and adult hormone treatment [$f(1,24) = 4.37$, $P < .05$] after the 175 mg/kg of NMDA injection (Fig. 1A). Morphine-exposed, OVX females displayed stereotypy significantly earlier than morphine-exposed, EB-injected female rats, and earlier than OVX controls. After the 200 mg/kg of NMDA injection, there was a main effect of drug [$f(1,24) = 7.45$, $P < .05$] and an interaction between drug and hormone [$f(1,24) = 4.42$, $P < .05$]. An EB injection in morphine-exposed females increased the latency to onset of stereotypy relative to all other groups. Neither prenatal drug exposure nor adult hormone treatment had any effects on stereotypy following a 150 mg/kg of NMDA injection.

In NMDA-induced clonic–tonic seizures, there was a main effect of hormone treatment after the 150 mg/kg of NMDA [$f(1,12) = 5.26$, $P < .05$] (Fig. 1B). All EB-injected females exhibited seizures later than OVX females. Neither prenatal drug exposure nor adult hormone treatment had any effects on clonic–tonic seizures after 175 or 200 mg/kg of NMDA. Additionally, there were no differences between saline- and morphine-exposed females when the incidences

of stereotypy or clonic–tonic seizures were examined after NMDA administration (data not shown).

3.2. KA-induced seizures

In KA-induced WDS, there was a main effect of prenatal drug exposure after the 15 mg/kg of KA [$f(1,35) = 38.99$, $P < .0001$]. Morphine-exposed females displayed WDS significantly later than controls, regardless of hormonal background (Fig. 2A). Neither prenatal drug exposure nor adult hormone treatment had any effect on the 10 mg/kg of KA injection-induced WDS. Additionally, there were no differences between saline and morphine exposure in incidences of WDS after KA administration in any of the groups (data not shown).

In KA-induced clonic seizures, there was a main effect of prenatal drug exposure [$f(1,18) = 10.33$, $P < .01$] and adult hormone treatment [$f(1,18) = 6.52$, $P < .05$] after 10 mg/kg of KA (Fig. 2B). Morphine-exposed females exhibited seizures significantly later than controls, regardless of hormonal background. Additionally, an EB injection decreased the latency to onset of KA-induced clonic seizures in both saline- and morphine-exposed female rats. There were no prenatal drug or hormone effects on KA-induced clonic seizures after 15 mg/kg. Additionally, the number of seizing OVX, EB-treated females was lower after prenatal saline than prenatal morphine exposure ($p < .05$) (data not shown).

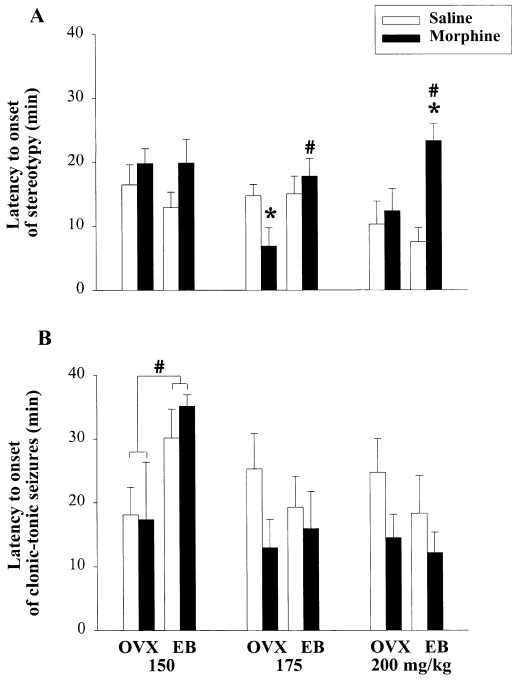

Fig. 1. Effect of prenatal morphine exposure on NMDA-induced seizures. Interaction between prenatal drug exposure and adult hormone treatment in NMDA-induced stereotypy (A) and a main effect of adult hormone treatment on NMDA-induced clonic–tonic seizures (B). Data are expressed as mean ± S.E.M. ($n = 10$). (A) $^{*}P < .05$ vs. saline-exposed females of the same hormone treatment. $^{\#}P < .05$ vs. OVX females of the same prenatal drug exposure. (B) $^{\#}P < .05$ vs. OVX females.

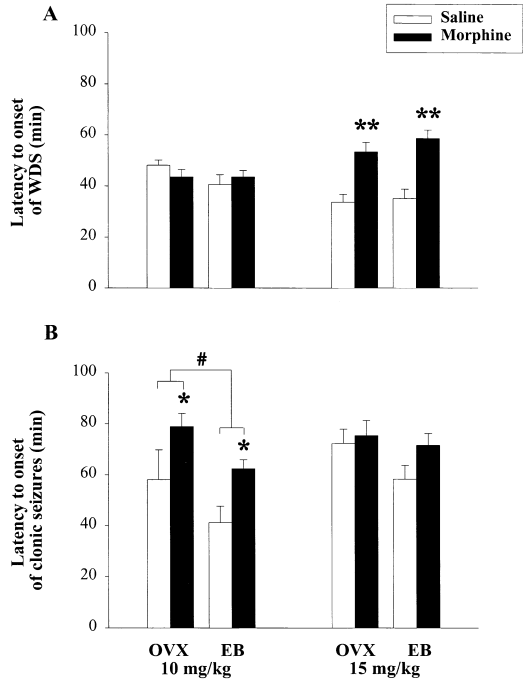

Fig. 2. Effect of prenatal morphine exposure on KA-induced seizures. A main effect of prenatal drug exposure on KA-induced WDS (A) and a main effect of prenatal drug exposure and adult hormone treatment on KA-induced clonic seizures (B). Data are expressed as mean ± S.E.M. ($n = 10$). (A) $^{**}P < .0001$ vs. saline-exposed females. (B) $^{*}P < .01$ vs. saline-exposed females. $^{\#}P < .05$ vs. OVX females.

4. Discussion

Our data demonstrate that prenatal morphine exposure has different effects on seizures and stereotypy induced by NMDA and KA. Prenatal morphine exposure increased the latency to onset of NMDA-induced stereotypy in EB-treated females, but had no effects on NMDA-induced seizures. These differential effects of prenatal morphine exposure on NMDA-induced stereotypy and clonic–tonic seizures were also observed in our previous study in adult male rats [15]. These results suggest that in both sexes, NMDA-dependent stereotypy and seizures are possibly under the control of different brain regions and/or neurotransmitters, and that these systems are differentially altered by prenatal morphine exposure.

On the other hand, prenatal morphine exposure increased the latency to onset of both KA-induced stereotypy and clonic seizures in adult female rats. This is not in agreement with our previous work examining adult, morphine-exposed males in the same experimental conditions. In morphine-exposed male rats, the latency to onset of KA-induced stereotypy was decreased without any effects on clonic seizures [15]. Differential effects of prenatal morphine exposure on seizure susceptibility in male and female rats have

already been demonstrated in some of our previous work [23–25]. Thus, the present and previous results suggest that prenatal exposure to morphine alters seizure susceptibility in a sex- and seizure model-dependent manner.

Additionally, our present study demonstrates that prenatal morphine exposure differentially alters estrogen regulation of NMDA- and KA-induced seizures in adult female rats. An EB injection increased the latency to onset of NMDA- but not KA-induced stereotypy in both drug-exposed and control female rats. It is interesting that an EB injection in both saline- and morphine-exposed females increased the latency to onset of NMDA-induced clonic–tonic seizures, and decreased it in KA-induced clonic seizures. Our findings that EB decreased the latency to onset of KA-induced seizures are in agreement with data of Woolley [27], where she showed an enhanced effect of estrogen on KA-induced seizures. Moreover, these data are also in agreement with some clinical work demonstrating that estrogen increases seizure susceptibility in women with epilepsy [12,13]. In contrast, an EB injection increased the latency to onset of clonic–tonic seizures in both saline- and morphine-exposed females after NMDA administration in our present study. These results may support the evidence, that NMDA and non-NMDA receptors are differentially altered by estrogen [14,28].

There may be a correlation between our work and that of Murphy and Segal [14] or Woolley and McEwen [28]. However, note that our work investigates the effects of steroid hormones on seizures, while the studies of Murphy and Woolley concentrate on estrogen-induced alterations in anatomical structures. Both Murphy and Segal [14] and Woolley and McEwen [28] showed higher number and density of dendritic spines and synapses in CA1 pyramidal cells [26,29], where NMDA binding sites are the most abundant, as a function of estrogen [11,26]. If estrogen-induced increases in NMDA binding sites correlates with a reduction in NMDA-induced clonic–tonic seizures as was seen in drug-exposed and control females, it would suggest that estrogen regulation of such effects are not influenced by prenatal morphine exposure. Subsequently, prenatal morphine exposure does not alter the anticonvulsant effects of estrogen on NMDA-induced seizure susceptibility. Thus, this could suggest that an acute estrogen administration-induced structural and/or functional changes may be separable and possibly controlled by separate mechanisms.

Additionally, the effects of an EB treatment on seizures were dependent on the dose of seizure-inducing drugs. An EB injection increased the latency to onset of seizures after the lowest dose of NMDA and decreased it after the lower dose of KA in both saline- and morphine-exposed female rats. Thus, estrogen regulation of seizure susceptibility is sensitive to the lowest but not the higher doses of both seizure-inducing drugs.

In contrast, an EB injection increased the latency to onset of stereotypy after the two higher doses of NMDA in morphine-exposed, OVX female rats, but did not change the latency to onset of WDS after KA administration in morphine-exposed, OVX females. However, the protective effect of EB on stereotypy appears to be more dependent on prenatal morphine exposure that increased the sensitivity to NMDA-induced stereotypy in the hormone-free females. Thus, prenatal morphine exposure could have shifted estrogen sensitivity of stereotypy mediated by NMDA, but not KA receptors. It is possible that prenatal morphine exposure rendered OVX and OVX, EB-injected females tolerant to KA-induced WDS. Thus, it may suggest that morphine exposure altered the drug sensitivity thresholds and the alterations are present only when an additional challenge is introduced to the animals such as the removal/replacement of the ovarian steroid estrogen.

Taken together, our data raise many questions about the long-term effects of prenatal morphine exposure and adult ovarian hormone regulation on stereotypy and seizure susceptibility of excitatory amino acids. However, they are difficult to interpret. One possible problem may be that the doses for NMDA and KA were too high for proper assessment of the effects of prenatal morphine exposure on estrogen regulation of seizures. We used doses for NMDA and KA that are known to effectively induce seizures [21,22]. Indeed, with these doses we observed high incidence of seizures, but they may have been too high to investigate estrogen regulation of seizures in female rats. Thus, we found alterations in seizure susceptibility only with the lower doses of both convulsants. Future studies may examine estrogen regulation of seizures with lower doses of NMDA and KA to detect differences in OVX, prenatally morphine-exposed females.

On the other hand, regarding the doses of NMDA or KA, one needs to use higher doses to investigate estrogen regulation on stereotypy. However, using higher doses to show differences in stereotypy may only be relevant to NMDA and not KA. Specifically, estrogen regulation of NMDA-induced stereotypy is more sensitive to gestational opiate exposure than KA-induced WDS. In fact, it seems that KA-induced WDS are not regulated by estrogen. Therefore, it is possible that the NMDA and non-NMDA receptor mediated estrogen regulation of seizures and stereotypy are differentially sensitive to prenatal morphine exposure. More studies are necessary to investigate the effects of prenatal morphine exposure on the interaction of CNS systems that are developing during mid to late gestation.

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