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Challenge dose of methamphetamine affects kainic acid-induced seizures differently depending on prenatal methamphetamine exposure, sex, and estrous cycle

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ABSTRACT

Even though it is obvious that glutamate plays an important role in the effect of psychostimulants on seizures, the role of non-NMDA receptors remains uncertain. The aim of the present study was to determine whether acute methamphetamine (MA) administration changes sensitivity to seizures induced with kainic acid in prenatally MA-exposed adult rats. Adult male and female rats (prenatally MA exposed, prenatally saline exposed, and controls) were divided into groups that received a challenge dose (1 mg/kg) of MA and groups that did not receive the MA challenge (saline injected). Systemic administration of 15 mg/kg kainic acid was used as a seizure model. Our results demonstrated that a single injection of MA (1 mg/kg) affects kainic acid-induced seizures differently depending on prenatal exposure, sex, and female estrous cycle. Even though daily injections of MA (5 mg/kg) in maternal rats did not have a long-term effect on susceptibility to seizures induced with kainic acid in adult progeny, sensitivity to the challenge dose of MA differed between the prenatal exposure groups.

1. Introduction

Studies in humans have demonstrated that in children of mothers who abuse psychostimulants, such as cocaine, amphetamine, and methamphetamine (MA), the occurrence of convulsions and epileptic seizures is increased [1–3]. These findings are further supported by experimental seizure models examining prenatally cocaine-exposed rats [4,5]. In addition, our previous seizure studies repeatedly demonstrated that prenatal MA exposure increases seizure susceptibility in adult male and female rats [6–8].

Because repeated administration of psychostimulants is known to induce sensitization [9], or in contrast tolerance [10,11], the aim of our most recent work was to investigate whether prenatal MA exposure induces changes in sensitivity to the same drug in adulthood. Our studies demonstrated that prenatal MA exposure changes behavioral sensitivity to the same drug in adulthood, perhaps in part because of the long-term changes in dopamine levels induced by prenatal MA exposure [12–14]. Our most recent studies further showed that the same prenatal MA exposure has a long-lasting effect on seizure susceptibility that is influenced by a single challenge dose of MA in adulthood [15,16]. Specifically, the response induced by an acute challenge dose of MA in adulthood differs between prenatally MA-exposed animals and their controls in flurothyl- and NMDA-induced seizure models.

Even though it is obvious that glutamate plays an important role in the effect of psychostimulants on seizures, it is still not clear which types of glutamate receptors are involved in this effect. Most studies, including our own, support the significance of NMDA receptors [7,8,16–21]. The role of non-NMDA receptors is, however, still uncertain. There are studies showing their importance in the effect of psychostimulants on seizures [4,20,22] and studies showing the opposite [8,18]. Even though our own study showed that NMDA receptors, but not non-NMDA receptors, play a role in the effect of prenatal MA exposure on seizures induced with NMDA and flurothyl in adult male rats [8], we were curious to learn if the challenge dose of MA in adulthood affects the susceptibility to kainic acid-induced seizures the same way in prenatally MA-exposed, saline-exposed, and control animals. Because previous studies have reported sex- and female gonadal hormone-induced differences in seizure susceptibility [23–25], both male and female rats were tested in the present study. In females, two contrasting phases of the estrous cycle, diestrus (low ovarian hormone level) and proestrus/estrus (high ovarian hormone level) [26], were compared.

2. Methods

2.1. Prenatal and postnatal animal care

Adult female Wistar rats (250–300 g) from Anlab Farms (Prague, Czech Republic) were randomly assigned to an MA-treated, saline-treated, or control group. MA (from the Faculty of Pharmacy of Charles University in Hradec Králové, Czech Republic) was diluted in distilled water and injected subcutaneously in a dose of 5 mg/kg throughout

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the entire gestation period (for details see [27]). Saline was injected subcutaneously at the same time and volume as MA. Control females were not given any injections. Day of delivery was counted as postnatal day (PD) 0. On PD 1, pups were cross-fostered so that each mother received the same number of pups with the same treatment and the same numbers of the pups with the other two treatments. The number of pups in each litter was adjusted to 12. The same numbers of male and female pups were kept in each litter. On PD 21, pups were weaned and housed in groups of the same sex. Animals were left undisturbed until adulthood. After reaching PD 60, animals were further divided into two groups: (1) those pretreated with MA challenge (1 mg/kg) and (2) those not given the MA challenge. Only one male and one female rat per group from each prenatal treatment were used from each litter to avoid litter effects.

2.2. Estrous cycle determination

Gonadally intact females were smeared by vaginal lavage to determine the phase of the estrous cycle. The smear was examined by light microscopy using 20× magnification. Two phases of the estrous cycle were recognized: diestrus (many leukocytes and very few cornified cells) and proestrus/estrus (some nucleated epithelial cells together with cornified cells with degenerated nuclei) [26]. When a female was in the appropriate phase of the estrous cycle, the experiment started. Each female was used only once.

2.3. Administration of MA challenge dose

To investigate sensitization or tolerance induced by prenatal MA exposure, a challenge dose (1 mg/kg sc) of MA was injected. The dose of 1 mg/kg was chosen based on the study of Suzuki et al. [9] and on our preliminary data (not shown) indicating that such a low dose does not induce stereotypy, but is often used for sensitization testing. The single injection of MA was administered 30 minutes prior to testing, when the behavioral response should approach the increased level [28].

In adulthood, all animals (control, prenatally saline exposed, and prenatally MA exposed) were randomly divided into groups that received the challenge dose of MA and control groups that received an acute saline injection. Thus, the following experimental groups of animals were tested for kainic acid induced-seizures, with and without acute MA pretreatment: males (control, prenatally saline exposed, prenatally MA exposed), females in diestrus (control, prenatally saline exposed, prenatally MA exposed), females in proestrus/estrus (control, prenatally saline exposed, prenatally MA exposed).

2.4. Induction of seizures with kainic acid

Kainic acid (Sigma–Aldrich, Czech Republic) was administered intraperitoneally in a dose of 15 mg/kg as in our previous study [8]. Kainic acid was dissolved in water, and 1 N NaOH was added to adjust the pH to 7.4. As described in the studies of Ben-Ari et al. [29,30] and Lothman and Collins [31], the progression of seizures after systemic kainic acid administration is as follows: a stereotypical sequence of staring spells, wet-dog shakes (WDS), and clonic seizures. WDS can be described as fast axial alternating head rotations that are expressed via granule cells of the dentate gyrus that are characterized by typical electroencephalographic phenomena [31–34]. Clonic seizures are characterized by rearing with bilateral upper extremity clonus, sporadic falling, and salivation that develops over 1–2 hours [29–31]. Clonic seizures do not end in death and usually recur within the 2-hour period. The present study recorded the incidence and latency to onset of WDS, the incidence and latency to onset of the first clonic seizure, and the length of the first clonic seizure within a 2-hour period after kainic acid administration.

2.5. Statistical analysis

There were 8–10 seizing animals per group. Statistical analysis was performed for males and females separately. A two-way ANOVA (prenatal exposure × challenge dose in adulthood) was used in male rats and a three-way ANOVA (prenatal exposure × challenge dose in adulthood × estrous cycle) in female rats. Bonferroni's test was used for post hoc comparisons. The incidence of seizures was analyzed using the χ^2 test. Differences were considered significant at $P<0.05$.

3. Results

3.1. Males

3.1.1. Incidence and the latency to onset of WDS

In adult male rats (Table 1), there was no effect on the incidence of WDS [$\chi^2=8.15$, $P=0.15$]. In the latency to onset of WDS, there was no main effect of prenatal exposure [$F(2, 54)=1.03$, $P=0.36$] or challenge dose in adulthood [$F(1, 54)=0.06$, $P=0.80$], but there was an interaction between prenatal exposure and challenge dose in adulthood [$F(2, 54)=3.22$, $P<0.05$]. Specifically, prenatal MA exposure, as well as prenatal saline exposure, increased the latency to onset of WDS in adult male rats given a challenge dose of MA, but did not do so in rats not given the adult challenge dose (Fig. 1A).

3.1.2. Incidence and latency to onset of clonic seizures

As can be seen in Table 1, there were statistical differences in the incidence of clonic seizures [$\chi^2=14.05$, $P<0.05$]. Specifically, the percentage of animals seizing after kainic acid administration was lower in control male rats given the MA challenge than in control males not given the MA challenge. In the other prenatal exposure groups, there were no MA challenge-induced differences in the incidence of seizures.

With respect to latency to onset of clonic seizures, there were main effects of prenatal exposure [$F(2, 49)=11.64$, $P<0.0001$], challenge dose in adulthood [$F(1, 49)=10.88$, $P<0.01$], and interaction between both prenatal exposures (saline and MA) and challenge dose in adulthood [$F(2, 49)=11.54$, $P<0.0001$]. Specifically, as shown in Fig. 1, rats exposed prenatally to MA ($P<0.001$) and saline ($P<0.0001$) both had a prolonged latency to onset of clonic seizures after a challenge dose of MA, whereas these prenatal exposure-induced differences were not apparent in males not given the acute MA challenge. The challenge dose of MA prolonged the latency to onset of clonic seizures in males prenatally exposed to saline ($P<0.0001$), but did not change it in other groups (Fig. 1B).

3.1.3. Duration of clonic seizures

As shown in Fig. 1C, there were no main effects of prenatal exposure [$F(2, 49)=3.03$, $P=0.06$] or challenge dose in adulthood [$F(1, 49)=0.07$, $P=0.79$] and no interaction between prenatal exposure and challenge dose in adulthood [$F(2, 49)=1.65$, $P=0.20$].

Table 1
Incidence of WDS and clonic seizures in adult male rats.

Prenatal exposure	Acute pretreatment	WDS	Clonic seizures
Control	Without MA	7/10 (70%)	9/10 (90%)
	With MA	12/21 (57%)	8/21 (38%)[a]
Saline	Without MA	12/12 (100%)	10/12 (83%)
	With MA	9/12 (75%)	10/12 (83%)
MA	Without MA	6/11 (55%)	8/11 (73%)
	With MA	14/18 (77%)	10/18 (56%)

Note. Values are occurrences of males with WDS and clonic seizures, respectively. Data are presented as occurrence of phenomenon/total number of tested males (percentage of occurrence).

[a] $P<0.05$ versus control males not given the MA challenge dose (χ^2 test).