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Prenatal Morphine Exposure Alters *N*-Methyl-D-Aspartate- and Kainate-Induced Seizures in Adult Male Rats

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ŠLAMBEROVÁ, R., L. VELÍŠEK AND I. VATHY. *Prenatal morphine exposure alters* N-*methyl*-D-*aspartate- and kainate-induced seizures in adult male rats*. PHARMACOL BIOCHEM BEHAV **65**(1) 39–42, 2000.—The purpose of the present study was to investigate whether prenatal exposure to morphine has effects on excitatory amino acid-induced seizures. Adult male rats, exposed on embryonic days 11–18 to saline or morphine, were injected with *N*-methyl-D-aspartate (NMDA) (150, 175, 200, 225, and 250 mg/kg) or kainic acid (KA) (15 or 20 mg/kg) in adulthood to assess the occurrence and latency to onset of stereotypy and seizures. The latency to onset of stereotypy was significantly increased after 175 mg/kg, and decreased after 200 mg/kg of NMDA in morphine-exposed animals. The lowest dose of NMDA (150 mg/kg) induced seizures in prenatally saline-treated control male rats but not in the morphine-exposed male rats. In the KA-injected group, prenatally morphine-exposed males had shorter latency to onset of wet-dog shakes, but there were no effects on the latency to onset of clonic seizures. The data suggest that prenatal morphine exposure has long-term effects on seizure susceptibility and the onset of stereotypy in the excitatory amino acid-induced seizure models. © 1999 Elsevier Science Inc.

Kainic acid NMDA Prenatal morphine Seizures Wet dog shakes

OPIATES and opioid peptides influence seizure susceptibility and sensitivity to convulsant drugs (9,14,23,28,31). Opiates such as morphine have both facilitatory (proconvulsant) and inhibitory (anticonvulsant) effects on seizures (9). The conditions under which morphine acts as a proconvulsant or anticonvulsant agent are not yet understood. However, it is known that the effects of morphine are dose dependent (9). Low doses of morphine usually have anticonvulsant effects (1,34), while high doses have proconvulsant effects (1). High doses of morphine increase the number of focal seizure episodes, duration of postseizure akinesis, and the incidence of generalized clonic seizures in the picrotoxin model of epileptic seizures (28). Moreover, high morphine doses given intrathecally elicit myoclonic seizures, and these seizures are not reversed by the opiate antagonist naloxone (14).

Repeated administration of morphine increases the sensitivity to seizures in adult rats (25). Chronic treatment with morphine enhances seizure susceptibility in amygdala kindling, electroshock-induced seizures, and several convulsant drug-induced seizures (22,25).

Epileptic seizures usually originate from an imbalance between neuronal excitation and inhibition (7,12). To rectify this imbalance, a suppression of neural excitation or potentiation of neural inhibition is necessary. These are mediated by excitatory amino acids such as glutamate and aspartate, and inhibitory amino acids such as γ-aminobutyric acid (GABA), respectively (7,12). Interestingly, both of these systems can be affected by morphine. Morphine influences GABA neurotransmission, via both GABA and benzodiazepine (BDZ) binding sites (5,9). Morphine has also effects on excitatory amino acid receptor subtypes, such as *N*-methyl-D-aspartate (NMDA) and kainic acid (KA) (1,11,15,34).

Administration of NMDA, a convulsant agonist of the NMDA subtype of glutamate receptor (26,33), induces stereotyped behaviors such as biting, and clonic–tonic seizures. Clonic–tonic seizures are manifested in adult rats as myoclonic convulsions of all four limbs and with tonic stretch of the limbs usually followed by death. Clonic seizures are associated with a loss of righting reflex (17). A single low dose of morphine inhibits NMDA-induced biting, and seizures (1,34). On the contrary, a single high dose of morphine potentiates NMDA seizures (1). Additionally, morphine withdrawal also

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activates NMDA neurotransmission and may induce seizures (15,27).

KA is an agonist of KA subtype of glutamate receptor (8,33) that has been frequently used as a model of limbic seizures (3,20). KA first induces stereotypy such as wet-dog shakes (WDS), and then clonic seizures, which are manifested in adult rats as clonic convulsions with preserved righting reflex (3). Morphine has multiple effects on KA-induced seizures. A subcutaneus (SC) injection of a low dose of morphine increases the incidence of convulsions and brain damage seen after KA-induced seizures (10). On the other hand, the incidence of KA-induced WDS, convulsions, and brain damage are reduced after SC administration of the nonspecific opiate antagonist naloxone (10). Interestingly, WDS may also be blocked by exogenous opiates, such as morphine, and endogenous opioid peptides (13,16); and morphine withdrawal, after chronic exposure, can also induce WDS (13). A single low dose of morphine administered intracerebroventricularly (ICV) induces WDS, rearing with bilateral forelimb clonus and generalized clonus (23). The underlying mechanism(s) of morphine effects on KA-induced seizures and WDS are not yet established, and may involve multiple neurotransmitter systems (21,32).

The above studies demonstrate that morphine has a wide variety of effects on neural excitation and inhibition involved in seizures. There is, however, no information available about susceptibility of prenatally morphine-exposed rats to seizures induced by excitatory amino acid analogues such as NMDA and KA. Therefore, the present study tested the hypothesis that exposure to morphine on embryonic days 11–18 alters susceptibility to seizures induced by systemic administration of NMDA or KA in young adult male rats.

METHOD

Eight-day pregnant Sprague–Dawley rats were purchased from Taconic Farms (Germantown, NY). Animals 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 11:00 h. Pregnant rats were randomly assigned to a morphine- (experimental) or a saline-treated (control) group. Morphine or 0.9% physiological saline injections were administered SC twice a day (0800 and 2000 h) on embryonal days 11–18 as described in the original work of Vathy et al. (30). The dose of the first three morphine injections was 5 mg/kg, and the dose of the remaining injections was 10 mg/kg (30).

The day of birth was counted as postnatal day 0 (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, all pups were weighed, sexed and crossfostered. Each mother raised half of her own and half of the adopted pups receiving the opposite treatment; whenever possible, an equal number of males and females were given to each mother of the opposite treatment (30). Litters were reduced to 10 pups. Pups were weaned on PND 25, and housed individually. Young adult male rats between age 60–70 days (n = 8–10) were used in these behavioral studies. Because we only use one animal from each litter to avoid litter effects, the remaining animals were assigned to other experiments.

Five different doses (150, 175, 200, 225, or 250 mg/kg) of NMDA dissolved in distilled water (50 mg/ml) were administered intraperitoneally (IP). Each animal was observed for 30 min after the NMDA administration. The incidence and latency to onset of stereotypy and clonic–tonic seizures were noted. The stereotypy involved sigmoidal tail movements (tail twisting), caudal biting, and enhanced rearing activity associated with orientation, jumping, and running. In some cases, NMDA administration was eventually lethal.

Different groups of morphine- and saline-exposed adult male rats were injected IP with 15 or 20 mg/kg of KA, dissolved in 0.01 M phosphate-buffer saline (PBS), pH 7.4. KA-induced WDS, scratching, washing, and clonic seizures were recorded for 1 h after KA administration. The incidence and latency to onset of WDS and clonic seizures were evaluated.

Planned comparisons included unpaired t-test for each NMDA or KA dose. Each morphine group was compared only once vs. the matching control group. A one-way ANOVA with Bonferroni/Dunn post hoc test was used for comparisons of dose-dependent differences in morphine- or saline-exposed animals. The level of significance in this case was automatically adjusted to the level corresponding to $p = 0.05$ for multiple comparison using the statistical package of StatView 5.0. Fishers' test was used for comparison of incidence of steretpypy and seizures between morphine- and saline-exposed animals. The level of significance was preset to $p < 0.05$.

RESULTS

NMDA-Induced Seizures

Stereotypy occurred after all doses of NMDA in both saline- and morphine-exposed male rats (Fig. 1A). Compared to saline-exposed rats, the latency to onset of stereotypy was significantly increased in morphine-exposed animals after an injection of 175 mg/kg NMDA ($p < 0.05$), but it was significantly shortened following an injection of 200 mg/kg NMDA ($p < 0.05$). Additionally, one-way ANOVA with Bonferroni/Dunn post hoc test determined that the onset of stereotypy in saline-exposed animals was significantly increased following the 200 mg/kg of NMDA compared to 175 mg/kg NMDA, $F(4, 24) = 1.16, p < 0.05$. In morphine-exposed animals, there was a significant reduction in the latency to onset of stereotypy after 200, $F(4, 30) = 1.98, p < 0.05$, and 225 mg/kg, $F(4, 30) = 1.98, p < 0.05$, of NMDA compared to 175 mg/kg.

As shown in Fig. 1B, in morphine-exposed males, the 150 mg/kg NMDA dose did not evoke any clonic–tonic seizures. There was a trend toward a shortened latency to onset of clonic–tonic seizures in morphine-exposed males compared to controls after the 200 mg/kg NMDA dose. However, the difference did not reach statistical significance. Using one-way ANOVA in saline-exposed males, the latency to onset of clonic–tonic seizures was increased following the injection of 200 mg/kg of NMDA compared to 150 mg/kg of NMDA, $F(4, 19) = 1.51, p < 0.05$.

Prenatal morphine exposure did not significantly alter the incidence of stereotypy and clonic–tonic seizures (data not shown).

KA-Induced Seizures

WDS and clonic seizures occurred after both doses of KA in saline- and morphine-exposed male rats. As shown in Fig. 2A, there was a significantly shorter latency to onset of WDS after the 15 mg/kg dose of KA in morphine- compared to saline-exposed males, $F(1, 10) = 16.28, p < 0.05$. There was no difference in the latency to onset of WDS after the 20 mg/kg dose of KA between morphine- and saline-exposed rats. There were no significant differences in the latency to onset of clonic seizures between saline- or morphine-exposed rats (Fig. 2B). Fi-

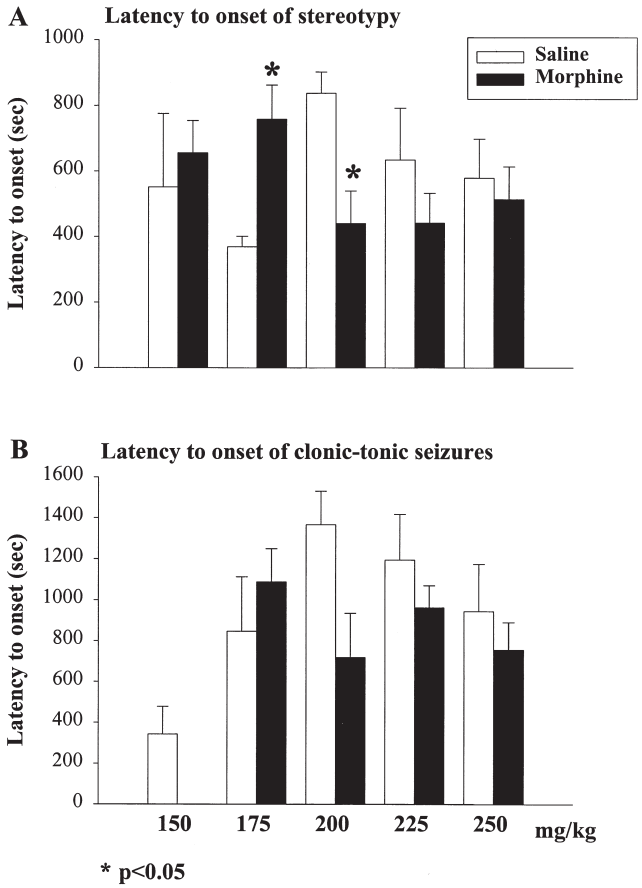

FIG. 1. Effects of prenatal morphine exposure on NMDA-induced stereotypy (A) and clonic–tonic seizures (B). x-axis: doses of NMDA; y-axis: latency to onset (mean ± SEM) of stereotypy (A), or latency to onset of clonic–tonic seizures (B). Asterisks indicate significant difference ($p < 0.05$) between morphine- and matched saline-exposed males using a *t*-test.

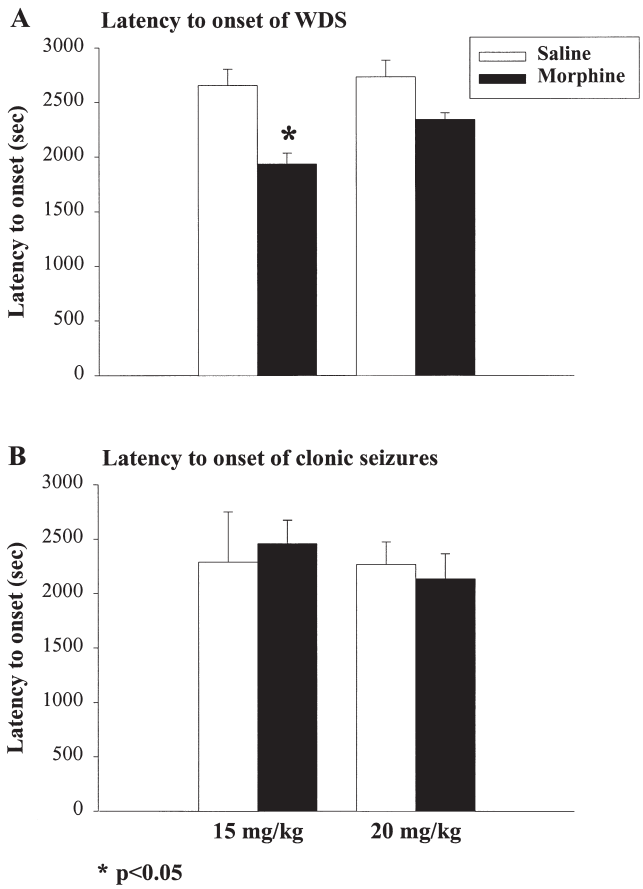

FIG. 2. Effects of prenatal morphine exposure on KA-induced WDS (A) and clonic seizures (B). x-axis: doses of KA. y-axis: latency to onset (mean ± SEM) of WDS (A), or latency to onset of clonic seizures (B). Asterisk denotes significant difference ($p < 0.05$) between morphine- and matched saline-exposed males using a *t*-test.

nally, prenatal morphine exposure also did not alter the incidence of WDS and clonic seizures (data not shown).

DISCUSSION

Our data demonstrate that prenatal morphine exposure in adult male rats enhances susceptibility to NMDA and KA-induced stereotypic behaviors. At low doses, morphine-exposed rats required more NMDA than controls to display stereotypic behavior. However, the latency values for the morphine-exposed animals display only modest changes over the entire NMDA dose range. The morphine effect appears to be more dependent on changes induced by the dose of NMDA on saline-exposed rats. The lowest dose of NMDA also induced clonic–tonic seizures in controls, but not in morphine-exposed animals.

Additionally, we have data indicating that in slices from the entorhinal cortex (EC) of morphine-exposed males, low Mg^{2+}-induced epileptiform activity was increased compared to saline-exposed controls (unpublished data). Decreasing Mg^{2+} in the extracellular space unblocks NMDA receptors. Thus, low Mg^{2+}-induced epileptiform activity originates from NMDA receptors activation. These in vivo and in vitro data indicate that effects of prenatal morphine exposure on seizures related to NMDA transmission may be dependent on NMDA receptor activation and/or on the NMDA dose. In addition, there may be also differences in the sensitivity of brain regions to NMDA. NMDA-induced seizures probably originate in hippocampal CA1 region where NMDA binding sites are the most abundant (6,19). These seizures generalize quickly (18), and NMDA receptors are important in this seizure generalization (24).

In adult male rats, prenatal morphine exposure activated KA-induced WDS, but had no effects on clonic seizures. This novel finding cannot be compared to other studies, because there are no reports in prenatally morphine-exposed rats on KA-induced seizures. However, different effects of morphine on KA-induced seizures and WDS were reported in several studies. Fuller et al. (10) demonstrated that low doses of morphine increase the incidence of convulsions and brain damage after KA administration, but there was no effect of morphine on KA-induced WDS. Differential effects of morphine exposure on KA-induced WDS and clonic seizures suggests that each may be under different neuronal control. On the other hand, a single low dose of morphine administered ICV induces WDS, rearing with bilateral forelimb clonus, and generalized clonus (23). Furthermore, opioid withdrawal is known

to induce WDS (13). It is possible that prenatally morphine-exposed rats experienced opioid withdrawal during development. Therefore, neural pathways controlling WDS may have been activated in utero so that these pathways may respond more readily to KA-induced WDS in adulthood. Unfortunately, mechanisms of WDS are not yet known.

Our data show that prenatal morphine exposure alters postnatal susceptibility to NMDA- and KA-induced seizures, suggesting an effect on the excitatory amino acid system. It is not to say that opioid receptor subtypes are not involved. Our experiments do not allow us to state that prenatal morphine exposure alters the excitatory amino acid modulated seizures either by μ, δ, and/or κ opioid receptors. There are studies showing that epileptiform features are mediated by δ, μ, κ, and σ opioid receptors (2,4,29). However, neither these nor the present studies assessed epileptiform activity using opioid peptide antagonist to examine the function of excitatory amino acid system. To make a definite statement about prenatal morphine exposure on excitatory amino acid-modulated seizures, more studies are required.

In conclusion, prenatal morphine exposure has different effects on stereotypy and seizures induced by excitatory amino acid agonists in adulthood. It is possible that prenatal morphine exposure does not directly influence NMDA or KA neurotransmission, but alters other neurotransmitter/neuromodulator systems that predominantly modify the stereotyped behavior but not the seizures. These modulatory systems may be altered by prenatal morphine administration. However, the nature of changes underlying these mechanisms needs to be established.

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