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Prenatal Morphine Exposure Induces Age-Related Changes in Seizure Susceptibility in Male Rats

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VATHY, I., J. VELÍŠKOVÃ AND S. L. MOSHÉ. *Prenatal morphine exposure induces age-related changes in seizure susceptibility in male rats.* PHARMACOL BIOCHEM BEHAV **60**(3) 635–638, 1998.—The purpose of this study was to investigate the effects of prenatal exposure to morphine (5–10 mg/kg on days 11–18 of gestation) on flurothyl seizure susceptibility in adult and developing male rats. In adult rats, prenatal morphine exposure increased the threshold to clonic seizures but not to tonic–clonic seizures. The effects of prenatal morphine exposure on clonic seizures were age dependent. At postnatal day (PND) 15, prenatal drug exposure did not alter the seizure threshold. At PND 25, there was a reduction in the threshold but by PND 38, the clonic seizure threshold was increased and this increase persisted into adulthood. Prenatal exposure to morphine did not alter the tonic–clonic seizure threshold in any age group of intact male rats. A group of male rats prenatally exposed to morphine was gonadectomized in adulthood. In gonadectomized rats both clonic and tonic–clonic thresholds were increased. These results suggest that exposure to morphine during mid to late gestation induces age-dependent alterations in the susceptibility to clonic but not tonic–clonic seizures. In adult male rats the threshold to tonic–clonic seizures is influenced by prior gonadectomy in adulthood. © 1998 Elsevier Science Inc.

Development Prenatal morphine Seizure susceptibility

THERE is ample evidence demonstrating that opiate exposure is associated with a variety of neurobehavioral disturbances. Over the last 2 decades, more than 300,000 infants, children, and adults have been exposed to opiates in early life in the US (41). The discovery of endogenous opioid systems that regulate somatic and neural growth raises the possibility that exogenous opiates, when present at inappropriate times or in nonphysiological concentration, can alter neural development. Thus, offspring exposed to an excess of exogenous opiates such as heroin or morphine as a result of maternal drug abuse may exhibit deleterious, long-term alterations in psychological, behavioral, and physiological processes when evaluated in adulthood. Understanding the consequences of prenatal drug exposure for the development of human central nervous system (CNS) and for adult behaviors is an extremely difficult task. Thus, studies in animals with a relatively short life span are essential to identify potential neurochemical and behavioral consequences of prenatal drug exposure.

Our previous work demonstrated that morphine exposure during mid to late gestation (on gestation days 11–18) induces permanent, sex-dependent changes in the brain of exposed adult rats. There are permanent alterations in all three (μ, δ, and κ) major opioid receptor subtypes in the hypothalamus, preoptic area, striatum, ventral tegmental area, cerebellum, and cortex (25), and in the catecholamine content and turnover in the hypothalamus (33,34). In addition, prenatal morphine exposure induced permanent changes in adult, gonadal steroid-dependent sexual behavior, which is influenced by both catechoalmines and opiates (32,33). These changes may constitute a substrate for altered seizure susceptibility.

Indeed, morphine and endogenous opioid peptides significantly influence seizure susceptibility in adult male animals. Systemic administration of opiates (especially those selective for μ and κ opioid receptors) suppressed seizures in several models (1–3,9–11,24,31). In addition, systemic administration of μ opioids induces postictal depression, a period of reduced

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seizure susceptibility immediately after a seizure that may prevent seizure recurrences (13,17,18,26,36). In contrast, intracerebral administration of endorphin, Met-enkephalin, and morphine have convulsant effects in adult rats (7).

Seizure susceptibility and the expression of seizures are age-specific phenomena. In humans, the incidence of seizures is highest during the first few years of life (16). In rats, ontogenetic studies demonstrated an increase in seizure susceptibility during the second and third postnatal weeks (15,23,27,35) and a decrease in seizure susceptibility between PND 30–35 just prior to adolescence (22). However, there is no information whether opiate exposure during gestation has an effect on seizure threshold in the offspring, and whether this effect is age specific, as suggested in studies with postnatal administration of PCP (28).

In the present study, we tested whether prenatal morphine exposure affects seizure susceptibility in male rats of various ages. Because several studies showed that in adult male rats, seizure activity can be affected by testosterone (20,29,30), to determine the influence of testosterone on seizure susceptibility, we also included a group of adult gonadectomized male rats prenatally exposed to morphine.

METHOD

Animals

Timed pregnant Sprague–Dawley rats were purchased from Taconic Farms (Germantown, NY) on the 8th day of conception. Pregnant females were housed individually in maternity pans with food and water available ad lib, and maintained on a 14 L:10 D dark cycle with lights off at 1200 h.

Materials

Morphine sulfate was obtained from the National Institute on Drug Abuse (Research Technology Branch, Rockville, MD), and was dissolved in physiological saline. Morphine and saline injections were administered subcutaneously (SC) in a volume of 0.1 ml.

Prenatal Drug Treatment and Weaning

Pregnant rats were randomly assigned to an experimental morphine-treated or a saline-treated control group. Both morphine and saline injections were administered SC two times a day (at 0800 and at 2000 h) from gestation day 11 through gestation day 18. The first three morphine injections were 5 mg/kg, while the other injections were 10 mg/kg as in our original study, which demonstrated long-term behavioral and neurochemical changes (32). The injection times coincide with the appearance of opiate (8) and steroid receptors (39,40). Control females received two daily injections of 0.9% NaCl (saline) on the same gestational days.

On the first postnatal day (PND 1) pups were sexed and weighed. The morphine-exposed pups were tattooed on one of their front foot pads with black India ink for identification, and all litters were reduced to a maximum of 10 pups. Morphine- and saline-exposed litters were crossfostered such that each mother raised half of her own and half of the adopted pups receiving the opposite prenatal treatment as in all our previous studies (32,33). Pups were weaned at PND 25, weighed, earpunched for identification, and housed individually with free access to food and water. Only one saline- and one morphine-exposed male from each litter was used in seizure experiments. The remaining animals were used in other experiments.

Seizure Testing

Male rats were tested for seizure susceptibility at various ages: PND 15, 25, 38, and adulthood (older than 60 days). Another group of prenatally treated adult male rats were gonadectomized 3 weeks prior to seizure testing. Different animals were tested at each age.

For seizure testing, the rats were challenged with flurothyl at a constant flow rate (20 μl/min) in an air-tight chamber (9.38 l) until tonic–clonic seizures occurred. Flurothyl is a convulsant ether that produces two types of seizures that occur sequentially; clonic seizures occur first, and tonic–clonic seizures follow. The two seizure types are mediated by differrent anatomical substrates and each seizure type is an end point by itself (4,5). Clonic seizures consist of facial and forelimb clonus (with or without tonic forelimb flexion) with preservation of the righting reflex; tonic–clonic seizures consist of loss of righting reflex, tonic flexion or extension of all four limbs, and then long clonus of all four limbs (37). The latency to the onset of first clonic and first tonic–clonic seizure was recorded. Because flurothyl was infused at a constant rate, the latency to onset of seizures allow for the calculation of the amount of infused flurothyl necessary to elicit the seizure, and to determine the flurothyl seizure threshold for the chamber size (in μl of flurothyl). An increased threshold denotes decreased seizure susceptibility for the particular seizure type. Seizure testing was performed between 0800 and 1300 h.

Statistical Analysis

The differences between morphine- and saline-treated, intact male rats during development were determined by two-way ANOVA (age × prenatal drug), and the Fisher's PLSD test was used for post hoc comparisons. The data between morphine- and saline-treated adult, gonadectomized male rats were analyzed by two-tailed unpaired *t*-test. The level of significance was preset to $p < 0.05$.

RESULTS

Prenatal morphine exposure did not produce any changes in locomotor activity in any of the age groups.

Effects of Prenatal Morphine Exposure on Seizure Susceptibility in Male Rats at Different Ages

There was a significant main effect of age on the clonic seizure threshold, $F(3, 48) = 16.30$, $p = 0.0001$. There was also a significant interaction between prenatal drug treatment and the age of the animals, $F(3, 48) = 6.46$, $p = 0.0009$. Because of the significant interaction between prenatal drug treatment and age of the animals, we additionally compared morphine- and saline-exposed rats within each age group using an unpaired *t*-test. Results of the *t*-tests demonstrated (Fig. 1) that at PND 15, the clonic seizure threshold was not affected by the prenatal morphine exposure. At PND 25, morphine-exposed rats had lower clonic seizure thresholds than controls ($p < 0.05$). At PND 38, morphine-exposed rats had higher clonic seizure thresholds than controls ($p < 0.05$). At PND > 60, morphine-exposed rats also had higher clonic seizure thresholds than controls ($p < 0.05$). There was a significant main effect of age on tonic–clonic seizure threshold, $F(3, 48) = 4.44$, $p < 0.05$. However, there was no significant effect of prenatal drug treatment on the tonic–clonic seizure threshold.

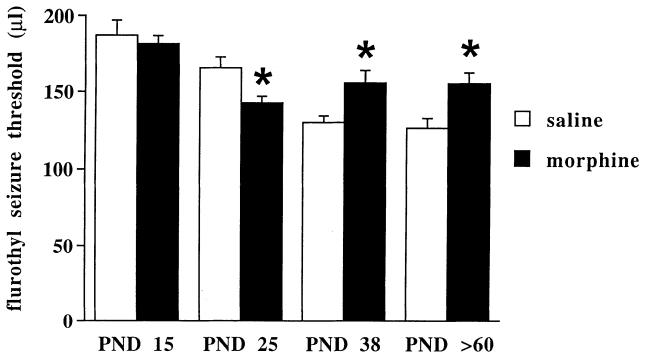

FIG. 1. Developing male rats: effects of prenatal morphine exposure on flurothyl-induced clonic seizures. Mean flurothyl seizure threshold values + SEM: asterisk indicates a significant difference ($p < 0.05$) compared to the control group (two-tailed unpaired *t*-test). In PND 15 rats (saline $n = 8$; morphine $n = 6$), prenatal morphine exposure has no effect on flurothyl seizures. In PND 25 rat (saline $n = 8$; morphine $n = 7$), prenatal morphine exposure has proconvulsant effects, while in PND 38 rats (saline $n = 7$; morphine $n = 7$), it has anticonvulsant effects on flurothyl seizures. In adult rats (saline $n = 7$; morphine $n = 7$), prenatal morphine exposure has proconvulsant effects.

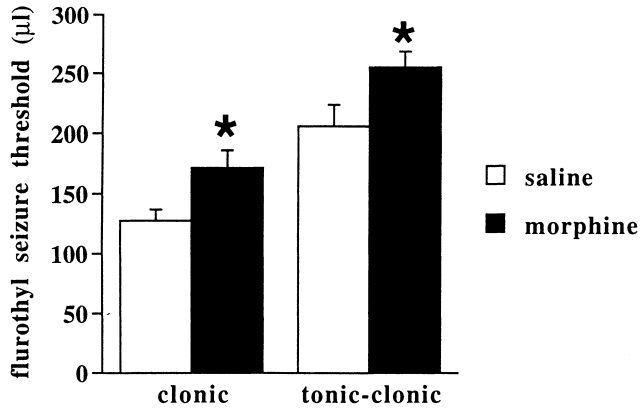

FIG. 2. Adult gonadectomized male rats: effects of prenatal morphine exposure on flurothyl-induced seizures. Mean flurothyl seizure threshold values + SEM: asterisk indicates a significant difference ($p < 0.05$) compared to the control group (two-tailed unpaired *t*-test). Prenatal morphine exposure has anticonvulsant effects on both clonic and tonic–clonic flurothyl seizures (saline $n = 8$; morphine $n = 9$).

Effects of Prenatal Morphine Exposure on Seizure Susceptibility in Adult Male Gonadectomized Rats

In gonadectomized adult male rats, prenatal morphine exposure altered seizure susceptibility (Fig. 2). Morphine-exposed gonadectomized rats had higher clonic seizure threshold than controls ($p < 0.05$). The threshold to tonic–clonic seizures was also increased ($p < 0.05$; Fig. 2).

DISCUSSION

Morphine during mid to late gestation produces long-term alterations in the CNS of exposed animals (25,33,34). This is further supported by the present study in terms of persistent changes in seizure susceptibility. We found that the effects of prenatal morphine exposure are age specific. Additionally, in gonadectomized adult male rats the thresholds for both clonic and tonic–clonic types of flurothyl seizures were affected, while in intact male rats, only the clonic seizure threshold was affected. Both seizure types have distinct substrates, and the findings in adult gonadectomized male rats further support the notion that different circuitry is required for the expression of clonic and tonic–clonic seizures (6).

Prenatal morphine exposure alters the density of hypothalamic μ opioid receptors (25). Prenatal morphine exposure also affects δ and κ opioid receptor density in many brain areas including the hypothalamus, preoptic area, striatum, cortex, ventral tegmental area, and cerebellum of adult animals (Vathy and Rimanóczy; unpublished). The alterations are site and receptor subtype specific. Therefore, it is likely that prenatal morphine exposure has a general effect on all three major opioid receptor subtypes, interfering with their binding characteristics in the brain of exposed animals subsequently inducing permanent alterations in several behaviors including seizure susceptibility. Anticonvulsant actions of morphine and enkephalins have been reported in animal models of reflex epilepsy and against PTZ- and flurothyl-induced seizures (12,14). Thus, prenatal morphine exposure may have modified seizure susceptibility by enhancing the anticonvulsant actions of endogenous opioids (12).

There is evidence that prenatal drug exposure is most detrimental to those systems that are developing at the time of drug administration (19). In the embryonic brain, the mid to late gestational morphine treatment coincides not only with the appearance of opioid receptors, but also GABA, catecholamine, and gonadal steroid receptors (19,38–40). Vathy and colleagues (33,34) demonstrated that prenatal morphine during the same gestational period induces alterations in hypothalamic NE content and turnover in exposed adult male and female rats, and that these alterations are sex dependent.

The effects of prenatal morphine exposure are age specific. We found that in PND 15 male rats, prenatal morphine exposure did not alter the threshold for clonic or tonic–clonic seizures compared to controls. At PND 25, in prenatally morphine-exposed rats, the threshold for clonic seizures was decreased compared to controls. In PND 38 and adult, prenatally morphine-exposed rats, the threshold for clonic seizures was increased compared to control rats. These age-dependent changes in seizure susceptibility by the prenatal morphine exposure suggest modulatory alterations in another age-specific process.

Testosterone levels phasically change with maturation, and may influence seizure susceptibility (21). The levels of testosterone fluctuate during development with a sudden decrease between PND 20–25 (21). Thus, in the present study we speculate that a decrease in testosterone level prior to PND 25 may be associated with the decrease of the threshold to clonic seizures, while the increase in testosterone level during adolescence may be related to the increased threshold observed in PND 38 and adult morphine-exposed rats. Once these postulated testosterone-related changes are induced in flurothyl threshold, they seem to be permanent, because gonadectomy in adulthood did not alter seizure susceptibility of gonadectomized rats to flurothyl. The flurothyl clonic seizure threshold was increased in both intact and gonadectomized, morphine-treated male rats. However, gonadectomy did affect the threshold to tonic–clonic seizures in morphine-exposed rats, although the changes in testosterone levels during devel-

opment did not affect the threshold to this type of seizure. Thus, the present data suggest that prenatal morphine exposure induces complex changes in the interactions between several CNS neurotransmitter/neuromodulator systems that alter seizure susceptibility as a function of age.

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