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Short communication

Prenatal stress affects 3,4-methylenedioxymethamphetamine pharmacokinetics and drug-induced motor alterations in adolescent female rats

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Abstract

We examined the influence of prenatal stress on 3,4-methylenedioxymethamphetamine (MDMA, 5 mg/kg p.o.) pharmacokinetics in adolescent female SD rats (30 days). Our results indicate that the metabolic rate of MDMA was higher in the prenatal stress group than in the control group. Moreover, MDMA-induced motor alterations were increased in prenatally stressed rats. These findings provide evidence that (i) prenatal stress increases sensitivity to MDMA, (ii) these effects are already detectable at the adolescent stage and (iii) early differences in metabolism may play a role in the behavioural changes associated with this drug of abuse.

Keywords: Maternal stress; Adolescence; MDMA; Pharmacokinetics; Behaviour; Individual variability

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") is a potent psychostimulant and widely used recreational drug of abuse (Parrott and Lasky, 1998; Gerra et al., 2002). In humans as well as in experimental animals, there is a high individual variability in the sensitivity to psychostimulants. Differences in psychostimulant pharmacokinetics may participate in the individual response to drugs (de laTorre et al., 2000).

An increased drug use and a higher risk of developing drug-related problems are often observed in the adolescent period (Laviola et al., 1999). Adolescent humans exhibit a reduced sensitivity to various drugs of abuse, and such insensitivity can promote greater use per occasion relative to that of more mature individuals.

In rodents, the animal model of adolescence (Spear and Brake, 1983) covers the whole postnatal period from weaning to adulthood (21–60 days) and represents a useful tool for investigating the effects of psychostimulants. Adolescent rodents exhibit elevated levels of novelty-seeking behaviour together with a peculiar sensitivity to cocaine (Laviola et al., 1995), amphetamine (Adriani et al., 1998) and MDMA (Morley-Fletcher et al., 2002).

In addition to age, environmental factors may also play a key role in determining individual variability to psychostimulants (Piazza and Le Moal, 1996). In rats, exposure to stress during prenatal life affects neurochemical systems that are particularly relevant to the study of drug abuse. Prenatal stress has been found to result in functional alterations of the mesolimbic system (Henry et al., 1995), enhanced propensity to self-administer amphetamine (Deminiere et al., 1992), as well as increased locomotor reactivity following nicotine administration (Koehl et al., 2000).

This study aimed to investigate the influence of prenatal stress on MDMA pharmacokinetics in female rats that were acutely administered this drug during adolescence. Animals were first evaluated in a simple motor coordination task and then were killed at different time points to determine MDMA levels in the blood. Our results indicate that

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prenatally stressed rats showed reduced elimination rates of MDMA and increased drug-induced motor alterations.

2. Materials and methods

2.1. Animals and breeding

Sprague–Dawley female rats weighing approximately 250 g, without prior breeding experience, were purchased from a commercial breeder (Charles River, Italy). Animals were housed in an air-conditioned room (temperature, 21 ± 1 °C, relative humidity 60 ± 10%), with a regular light–dark cycle (lights on at 0800 h). Water and food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, Italy) were available ad libitum. Females were placed with a sexually experienced male, and vaginal smears were inspected daily until the discovery of spermatozoids (designated day of gestation 0), after which, they were housed individually in Plexiglas cages (30 × 20 × 15 cm). Pregnant females were then randomly assigned to prenatal stressed or control groups. The protocol of the experiment strictly followed the European Community guidelines for the use of experimental animals.

2.2. Prenatal stress

Stress procedure was conducted according to Maccari et al. (1995). On day 11 of pregnancy until delivery on day 21, pregnant females were submitted daily to three stress sessions starting at 0900, 1200 and 1700 h during which they were placed in plastic transparent cylinders (diameter = 7 cm; length = 19 cm) and exposed to bright light for 45 min. Control pregnant females were left undisturbed in their home cages. Offspring was weaned on day 21 after birth, and only offspring from litters containing 10–14 pups with a comparable number of males and females were used in the experiments. After weaning, animals from each experimental group were housed in same-sex litter groups of five and kept under the same environmental conditions throughout the experiment. In the present study, only females of the control and prenatal stress groups were used ($n = 30$ each group).

2.3. Drug treatment

3,4-Methylenedioxymethamphetamine hydrochloride (Lipomed, Switzerland) was diluted in water to provide the appropriate dose (5 mg/kg) and administered orally 1% of body weight. Dosage was selected on the basis of literature (Miczek and Haney, 1994). Adolescent female rats (30 days of age) from control and prenatal stress groups were weighed and gavaged once with either water (vehicle subjects) or MDMA. Vehicle-treated subjects were tested immediately after the gavage procedure, whereas MDMA-treated subjects were tested at different time points such as 15, 60, 120 or 180 min after the gavage. Within litters, each rat was randomly assigned to one of the five time points after treatment (t0, t15, t60, t120 and t180).

2.4. Motor coordination test

Rats were trained to traverse a straight Plexiglas runway (width = 5 cm; length = 100 cm) suspended 1 m above the floor (adapted from McFarland and Ettenberg, 1998). The apparatus was located in a sound-attenuated room, and the test was conducted under dim light. The test consisted of two trials of 2 min each with a 1-min intertrial interval. The animal was gently placed on one end (departure), and the following measures of impaired balance were recorded: episodes of inactivity on the runway, frequency of slips (the animal slips on the runway with one fore paw) and of twists (the animal turns completely on its body with the four paws).

2.5. Pharmacokinetics of MDMA

At the end of the behavioural tests, the animals were killed by decapitation, and trunk blood was collected in heparinised vials. MDMA concentration was then measured in 50-μl blood samples by headspace solid-phase microextraction tandem gas chromatography–mass spectrometry (Gentili et al., 2002). This method provides good sensitivity and specificity with limits of detection and of quantitation below 1 and 2 ng/ml, respectively. Intra- and inter-assay precision were within 2% and 9%, respectively.

2.6. Statistics

Data were analysed using parametric analysis of variance (ANOVA) with two levels of group (control vs. prenatal stress) as between-group variable and five time points (t0, t15, t60, t120 and t180) as within-group variables. Planned

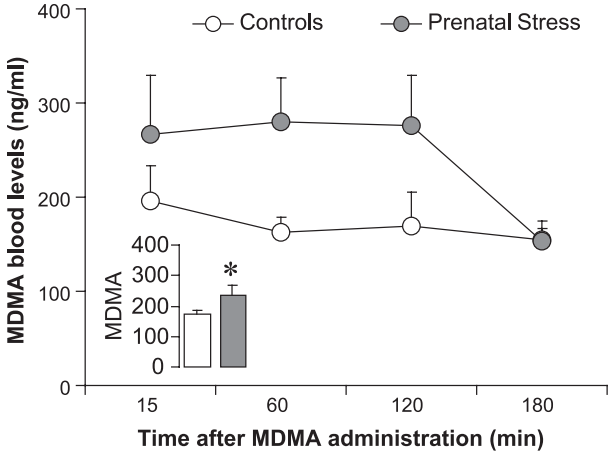

Fig. 1. Pharmacokinetic analysis of MDMA (5 mg/kg, p.o., acute) blood levels in adolescent prenatally stressed and control female rats (30 days of age). MDMA levels are expressed as nanograms per milliliter. Each point represents the mean ± S.E.M. for six animals. Inset: same data pooled over the time points variable. *$p < 0.05$.

contrast analysis followed when appropriate. Significance was set at $p < 0.05$.

3. Results

3.1. MDMA pharmacokinetics

Results are presented in Fig. 1. Prenatally stressed rats had higher concentrations of circulating MDMA than did controls [ANOVA, main effect of group, $F(1,8) = 5.87$, $p < 0.05$, see inset]. A trend towards a time-dependent decrease in levels of MDMA was also observed (main effect of time just missing significance [$F(3,24) = 2.62$, $p = 0.07$]). At t180, blood MDMA levels no longer differed between the groups.

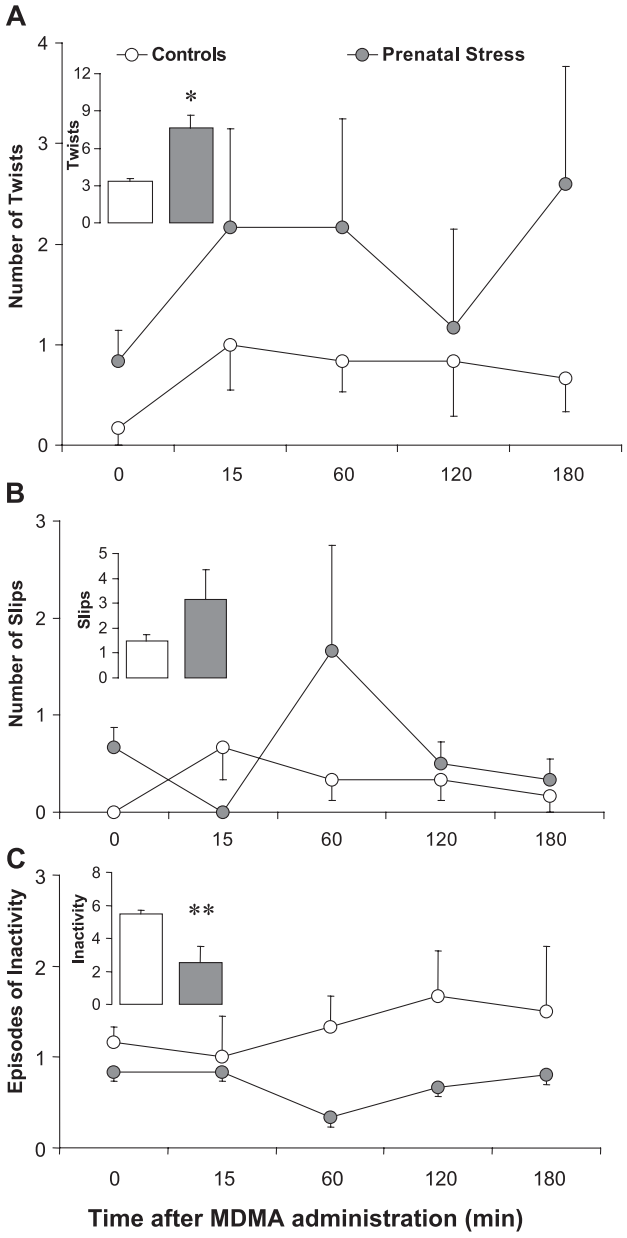

Fig. 2. Motor alterations induced by acute MDMA (5 mg/kg, p.o.) in prenatally stressed and control adolescent female rats (30 days of age) on number of twists (A), number of slips (B) and episodes of inactivity (C). Each point represents the mean ± S.E.M. for six animals. Insets: same data pooled over the time points variable.*$p < 0.05$; **$p < 0.01$.

3.2. Motor test

Results are presented in Fig. 2. ANOVA yielded a main effect of group (see insets) for number of twists [$F(1,9) = 4.94$, $p < 0.05$, Fig. 2A] and episodes of inactivity [$F(1,9) = 10.48$, $p < 0.01$ Fig. 2C], with prenatally stressed rats making more twists than controls, whereas the opposite was found for episodes of inactivity. An effect of group just missed significance [$F(1,9) = 2.15$, $p = 0.07$] but revealed a tendency for the prenatally stressed animals group to make more slips than the controls (Fig. 2B). As a whole, no main effect of time was observed on any of the behavioural parameters.

4. Discussion

In the present study, prenatal stress increased plasma levels of MDMA with respect to control levels and induced a higher frequency of altered motor coordination following MDMA administration, thus indicating a strong consistency between drug blood levels and behaviour. Moreover, the blood concentrations of MDMA in adolescent rats were already within the range reported following a single MDMA administration in humans (Helmlin et al., 1996), thus supporting the periadolescent rodent as a valid animal model to be used in the assessment of the vulnerability to psychostimulants.

The constant recovery of MDMA in the prenatal stress group seems to point towards an inhibition of MDMA metabolism. Differences in the metabolism of MDMA have been reported in humans (Hiramatsu et al., 1990) as well as in animals (Malpass et al., 1999). These differences may be due to changes in enzyme activity. Interestingly, the prenatal stress group showed a sharp drop in the levels of MDMA 3 h after drug administration, although behavioural alterations were still present. This difference could be due to differences in the metabolism of drug in the blood and in the brain, a point that should be taken into consideration in future studies. Given the importance of drug metabolism in determining the magnitude of the effects of a drug (de laTorre et al., 2000), the differences in MDMA metabolism observed may affect the likelihood of adverse consequences in prenatally stressed rats. One direct consequence would be the development of acute toxicity at moderate doses of MDMA, because the drug would accumulate in the body instead of being metabolised and inactivated.

It has been suggested that, in addition to the serotonergic system (Ricaurte et al., 2000), the corticotropic axis plays a role in the neurotoxic effects of MDMA. Thus, MDMA

stimulates corticosterone release (Nash et al., 1988) and alters corticoid receptor gene expression (Yau et al., 1994). Prenatal stress is known to induce a long-lasting impairment of feedback inhibition of the corticotropic axis (Maccari et al., 1995) with prolonged stress-induced corticosterone secretion in adult (Vallee et al., 1996) as well as adolescent (Morley-Fletcher et al., 2003) rats. In addition, prenatal stress induces an impairment of the serotonergic system (Peters, 1990). These data strongly suggest that corticotropic as well as serotonergic activity plays a key role in the enhanced response to MDMA found in prenatally stressed rats.

Our study provides further evidence that prenatal stress increases vulnerability to drugs. Exposure to early stress results in long-term functional alterations of the mesolimbic system (Henry et al., 1995) and enhanced vulnerability to a variety of psychostimulants, such as amphetamine (Deminiere et al., 1992), nicotine (Koehl et al., 2000) and MDMA, at the behavioural and pharmacokinetical level. This animal model appears to be useful for investigating individual variability in the response to such drugs during adolescence.

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