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Sex differences in methamphetamine pharmacokinetics in adult rats and its transfer to pups through the placental membrane and breast milk

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ABSTRACT

Background: Methamphetamine (METH) abuse is a growing health problem worldwide, and METH use during pregnancy not only endangers the mother's health but also the developing fetus. To provide better insight into these risks, we performed the following experiments.
Method: First, we investigated how sex influences the pharmacokinetics of METH and amphetamine (AMP) in male and female rats. Subsequently, we simulated chronic exposure of prenatal infants to METH abuse by investigating brain and plasma levels of METH and AMP in dams and pups. Finally, we modeled chronic exposure of infants to METH via breast milk and investigated sex differences in pups with regard to drug levels and possible sensitization effect of chronic prenatal METH co-treatment.
Results: We observed significantly higher levels of METH and AMP in the plasma and brain of female rats compared to males. Additionally, brain concentrations of METH and AMP in pups exposed to METH prenatally were equivalent to 62.13% and 37.78% relative to dam, respectively. Plasma concentrations of AMP where equivalent to 100% of the concentration in dams, while METH was equivalent to only 36.98%. Finally, we did not observe a significant effect relative to sex with regard to METH/AMP levels or sensitization effects linked to prenatal METH exposure.
Conclusion: We demonstrated that female rats display higher levels of METH and AMP, thus indicating a greater risk of addiction and toxicity. Furthermore, our data show that pups are exposed to both METH and AMP following dam exposure.

1. INTRODUCTION

Methamphetamine (METH; 2-methylamino-1-phenylpropane) is a strong stimulant drug inducing vigilance, increased physical activity, decreased appetite, increased respiration, hyperthermia and euphoria. The metabolism of METH has been well described (Caldwell et al., 1972; Kanamori et al., 2005; Maurer et al., 2000). Briefly, METH is *N*-demethylated to its psychoactive metabolite amphetamine (AMP) or hydroxylated to *p*-hydroxymethamphetamine. METH is mainly excreted in the urine, either in unchanged or metabolized form. The ratio between excreted METH and its metabolites, as well as the elimination half-life, varies among species (Caldwell et al., 1972). Investigations have shown that rats eliminate METH much faster ($t_{1/2}$ = 70 min) than human ($t_{1/2}$ = 12 h) (Cho et al., 2001) and that rats produce 2–3 times more AMP than humans (Rivière et al., 1999, 2000). The elimination of METH and AMP is partially dependent on urinary pH. Alkaline urine facilitates reabsorption of the drug, which delays renal clearance and increases the elimination half-life from blood or plasma (Anggard et al., 1973). Variations between species, as well as sex differences in METH pharmacokinetics, have to be taken into account when interpreting preclinical data.

The similarity of the chemical structure of METH to monoamine neurotransmitters (dopamine and noradrenaline) determines its

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mechanism of action (Sulzer et al., 2005; Heal et al., 2013). METH affects the function and trafficking of dopamine transporters (DAT) and vesicular monoamine transporters, specifically transporter-2 (VMAT-2), leading to a reversal of DAT transport function. It leads to increased release of dopamine from presynaptic terminals (Sulzer et al., 1995), as well as synaptic vesicles (Sulzer et al., 1995). This mechanism is complemented by inhibition of dopamine reuptake and inhibition of monoamine oxidase (MAO) A and B subtypes, with higher selectivity for MAO A than MAO B (Scorza et al., 1997; Robinson, 1985).

Elevated extracellular concentrations of dopamine lead to increased stimulation of dopaminergic receptors in the mesocorticolimbic (mesolimbic) pathway of the brain. The pathway originates in the ventral tegmental area and projects to the nucleus accumbens, the medial prefrontal cortex and other forebrain regions (Robinson and Becker, 1986; Pierce and Kumaresan, 2006). This pathway is considered a major neuronal circuit responsible for reward processes and addiction.

Worldwide, the abuse of METH is a serious health problem. Approximately half of all METH users are women, mostly of reproductive age, and consequently some of them become pregnant while using the drug (Cohen et al., 2007). METH crosses the placental and hematoencephalic barriers easily (Burchfield et al., 1991; Dattel, 1990) and therefore it can affect the development of the fetus. Moreover, the use of amphetamines during pregnancy may disrupt the physiological function of the placenta, thus further damaging the developing fetus (Ganapathy et al., 1999). Additionally, it is known that amphetamines are potent competitive inhibitors of placental serotonin and norepinephrine transporters (Ramamoorthy et al., 1995). This could play an important role in amphetamine fetotoxicity by leading to an increase in the concentrations of vasoactive monoamines, serotonin and norepinephrine, in the intervillous space. Studies suggest that METH exposure during pregnancy can impair the development of the neonatal central nervous system (Williams et al., 2003; Slamberova et al., 2006). Smith et al. (2008) observed decreased arousal, increased stress and poor quality of movement in prenatally METH-exposed neonates. Another study demonstrated altered neonatal behavior patterns characterized by abnormal sleep, poor feeding, tremors, and hypertonia, as well as visual and motor difficulties in neonates exposed to METH during the prenatal period (Oro and Dixon, 1987). In addition to in utero exposure, suckling neonates can also be exposed to METH postnatally since amphetamines are concentrated and secreted in the mother's breast milk (Steiner et al., 1984) and subsequently consumed by the offspring.

Our previous studies demonstrated that maternal injections of METH (5 mg/kg) in female rats during gestation and/or lactation periods impair maternal behavior (Slamberova et al., 2005a,b) and resulted in delayed postnatal sensorimotor development of their offspring (Slamberova et al., 2006). The impairment effect on pup development can be decreased or suppressed by postnatal fostering using a control dam (Hruba et al., 2009). In a previous study we showed that prenatal METH exposure has long-lasting effects on behavior, cognition, pain sensitivity and seizure susceptibility (Slamberova, 2012) and that prenatal METH exposure affects sensitivity to the same or similar drugs in adulthood (Slamberova et al., 2013a,b). To investigate whether our behavioral data correspond to METH and AMP levels in the brain and blood, the present study was planned.

In light of these previous findings, the first goal of this study was to investigate whether there are sex differences in METH and AMP levels in the brain and plasma following acute exposure to METH in adult rats. Second, we wanted to simulate chronic prenatal exposure to METH similar to that which might be experienced by fetus in drug-abusing women. To assess this, we investigated brain and plasma levels of METH and AMP in pups (gestational day, GD 21) from dams treated with METH during gestation (GD 1–GD 21). Third, we investigated the effect of chronic exposure to METH through consumed breast milk by comparing drug levels in male and female pups. Last, we examined possible sensitization effects associated with chronic prenatal METH exposure on METH and AMP levels in newborn pups followed by continued chronic exposure to METH via breast milk.

2. Materials and methods

2.1. Chemicals

Chemicals and reagents were of commercial origin: D,L-amphetamine (Cambridge Isotope Laboratories, UK), D,L-methamphetamine (Cambridge Isotope Laboratories, UK), hydrochloric acid (37%; Sigma Aldrich, USA), ethylenediaminetetraacetic acid (=EDTA; 99.99%; Sigma Aldrich, USA), water (LC–MS grade; Fluka, Switzerland), methanol (LC–MS grade; Fluka, Switzerland), acetonitrile (LC–MS grade; Fluka, Switzerland). Isotopically labeled standards were acquired commercially as indicated: D,L-amphetamine-d_5, 98 atom % D (Cambridge Isotope Laboratories, UK); D,L-Methamphetamine-d_5, 98 atom % D (Cambridge Isotope Laboratories, UK). D-methamphetamine hydrochloride (purity > 98%; Sigma Aldrich, USA) was used for animal injections in all experiments.

2.2. Animals

Male and female adult Wistar rats (weighting from 250 to 350 g) were delivered by Anlab (Prague, Czech Republic) from Charles River Laboratories (Germany). For one week before use, the rats were kept in transparent Plexiglas cages measuring 25 × 25 × 50 cm located in an air-conditioned animal room with a 12:12 h light–dark cycle (lights on at 6:00 a.m.). Animals had free access to water and food. All experiments were done in accordance with European Union regulations on animal care and protection, the Animal Protection Code of the Czech Republic and NIH guidelines. The procedures for animal experimentation utilized in this report were reviewed and approved by the Institutional Animal Care and Use Committee of the Third Faculty of Medicine, Charles University, Prague, CZ and were in agreement with Czech Government Requirements relative to its Policy of Humane Care of Laboratory Animals (No. 246/1992) and with the regulations of the Ministry of Agriculture of the Czech Republic (No. 311/1997). In all experiments, animals were euthanized by decapitation without anesthetics.

2.3. Drug administration and sample collection

Comparison of METH and AMP pharmacokinetics in male and female rats. On the day of the experiment male and female rats were divided into groups based on their scheduled time of sacrifice (0, 10, 20, 30, 40, 50, 60, 180 min and 6, 9, 12 and 24 h post-injection) and administered with a single dose of 1 mg/kg or 5 mg/kg, s.c. of METH dissolved in water. Three male and three female rats were used for each time-point group and dose. A total of 144 animals was used. The animals were weighed before injection and the dose of METH was adjusted accordingly. All animals received the same volume of liquid per 1 kg of body weight.

Analysis of maternal and fetal concentrations of METH and AMP following chronic exposure of dams to METH. The conception procedure was conducted using the same method as in our previous experiments (Slamberova et al., 2005a,b). Adult females were smeared by vaginal lavage to determine the phase of the estrous cycle. The smear was examined using a light microscope. At the onset of the estrous phase of the estrous cycle (Turner and Bagnara, 1976), each female rat was housed with one sexually mature male overnight. The next morning females were smeared for the presence of sperm and returned to their previous home cages. This was counted as gestational day (GD) 1. On GD 1 the daily injections started and continued to the day of delivery. Pregnant females were administered METH (5 mg/kg/day, s.c.) or saline (at the same time and volume, s.c.). On GD 21 (one day before expected delivery) females were sacrificed, the dam's blood and brain were collected and the pups were immediately removed. Blood and brains were collected from the pups as well. Three pregnant female rats treated with METH and six of their pups were used in the study. One saline treated pregnant female rat and two of her pups were used as a negative control. A total of 12 animals was used. As expected, METH and AMP levels in saline-exposed animals were immeasurable. The tissue from saline-exposed animals was utilized for the development of the LC–MS/MS method.

Investigation of transferal of METH and AMP to pups via *breast milk.* Ten adult female rats were fertilized (see above) and injected daily with METH (5 mg/kg, s.c., $n = 5$) or saline (1 mL/kg, s.c., $n = 5$) throughout the entire gestation (GD 1–22) or gestation and lactation (postnatal day, PD 1–21) periods. On postnatal day (PD) 1, pups were cross-fostered, so each mother (saline- or METH-treated) fostered part of her own and part of the pups from mother with the opposite treatment. Four treatment groups were established: (1) (NaCl/NaCl) pups exposed prenatally to saline and fostered by a saline treated dam, (2) (NaCl/METH) pups exposed prenatally to saline and fostered by a METH treated dam, (3) (METH/NaCl) pups exposed prenatally to METH and fostered by a saline treated dam, (4) (METH/METH) pups exposed prenatally to METH and fostered by a METH treated dam. Pups were not injected as part of