Effects of Neonatal Exposure to Methamphetamine

Catecholamine Levels in Brain Areas of the Developing Rat

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ABSTRACT: **Neonatal exposure to moderate doses of methamphetamine during the first month of life in the rat affects tyrosine hydroxylase gene expression in the substantia nigra and nigrostriatal tyrosine hydroxylase activity. The main goal of this work was to evaluate the ontogeny of the neurochemical effects of repeated exposure to moderate doses of methamphetamine during the first month of life in the rat. Norepinephrine, dopamine, and dihydroxyphenylacetic acid levels were measured in target areas of methamphetamine: the substantia nigra, ventral tegmental area, caudate-putamen, nucleus accumbens, and medial prefrontal cortex. On postnatal day 1 (PND1), Wistar rat litters, culled to eight pups, sex balanced, were randomly attributed to either methamphetamine or control groups. Methamphetamine groups were administered 10 mg of (±)-methamphetamine/kg body weight/day, subcutaneously, from PND1 until the day prior to sacrifice; control groups received isovolumetric saline. Groups were sacrificed on PND7, PND14, and PND30. Neonatal methamphetamine exposure increased norepinephrine levels in the substantia nigra of PND30 rats; on PND14, this variation was evident only in male pups. In the substantia nigra, the dihydroxyphenylacetic/dopamine ratio was also affected in PND30 males. In the ventral tegmental area, catecholamine levels were not affected by methamphetamine. Norepinephrine levels were also increased in the caudate-putamen of PND7 male and PND14 female methamphetamine-exposed pups and in the nucleus accumbens of PND14 female and PND30 male and female pups. Catecholamine levels in the medial prefrontal cortex were not affected by neonatal methamphetamine administration.**

KEYWORDS: **methamphetamine; rat; Wistar rat; neonatal; dopamine; norepinephrine; dopaminergic areas; gender**

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INTRODUCTION

Methamphetamine (Meth), a methylated analogue of amphetamine, acts upon the catecholaminergic systems of the central nervous system, namely upon the dopaminergic and noradrenergic systems (see, for example, Brunswick *et al.*[1] and Wang *et al.*[2]). In the rat, the development (and eventual maturation) of the catecholaminergic systems starts in prenatal life, and proceeds in the neonatal period, from the day of birth until the end of the first month of life.[3–5] In adult rats, chronic administration of a high dosage (20 mg/kg, intraperitoneally, every 12 h for 10 days) of Meth was shown to selectively damage dopaminergic pathways, namely the nigrostriatal[6] and the mesocorticolimbic pathway.[7] The place of origin of the dopaminergic neurons of the nigrostriatal and of the mesocorticolimbic pathways lies mainly in the substantia nigra pars compacta and in the ventral tegmental area, respectively, and their main sites of termination are, respectively, the caudate-putamen and the nucleus accumbens and frontal cortex. In particular, Meth was shown to act preferentially upon the sites of termination of dopaminergic pathways, such as the caudate-putamen, the nucleus accumbens, and the medial prefrontal cortex, whereas the places of origin of the dopaminergic neurons, the substantia nigra, and the ventral tegmental area are relatively spared.[8,9] The effects of Meth exposure throughout the first month of life in the rat are not yet completely characterized, but they may provide an insight about the potential effects of persistent exposure to Meth during the last trimester of human pregnancy, because some parallelism may be found between the development of the central nervous system of rats and humans, in these respective periods.[10] This work aimed to provide further details of the potential effects of neonatal Meth exposure in the rat. To this end, on key ages (PND7, PND14, and PND30), male and female pups were assessed for catecholamine levels in areas of the dopaminergic system implicated in the generation of pleasure and development of addiction.[11,12]

MATERIAL AND METHODS

Animals, Treatments, and Sampling

Nulliparous Wistar female rats (60 days old), purchased from the colony of the Gulbenkian Institute for Science, Oeiras, Portugal, were bred in the Institute of Anatomy, Medical School of Porto. Institutional guidelines were followed for animal care. The animals were kept in a room with controlled photoperiod (7:00 AM–7:00 PM) and temperature (22 ± 1°C). At the onset of breeding, adult females (over 8 weeks old) were placed with males from 8:00 PM to 8:00 AM. Females had *ad libitum* access to water and food, and pregnancies were carried to term. After the first meal, pups were individually weighed and assessed for any gross alterations, and litters were culled to eight pups (four males and four females, whenever possible). Pups were individually marked, and litters were randomly attributed to Meth or control groups.

Pups were injected subcutaneously, twice a day (8:30–9:00 AM, 5:00–6:30 PM), from postnatal day 1 (PND1) until the day before sacrifice, with 10 mg/kg body weight/day of (±)-methamphetamine hydrochloride (Sigma Chemical, St. Louis, MO) dissolved in a 0.9% saline solution. Control groups received saline, isovolu-

metric to Meth. Pups were weaned on PND21. Rats were sacrificed by decapitation on PND7, PND14, and PND30.

The whole brain was quickly removed, frozen by immersion in 2-methylbutane over dry ice, and kept at –80°C until assayed.

Sample Preparation and Catecholamine Detection

Catecholamine levels were measured, by high-performance liquid chromatography (HPLC) with electrochemical (EC) detection in brain areas of PND7, PND14, and PND30 pups. On PND7, the determinations were performed in the nucleus accumbens, caudate-putamen, and ventral mesencephalon (comprising the substantia nigra, the ventral tegmental area, and possible contamination with non-dopaminergic areas), whereas on PND14 and 30 the neurochemical measurements were conducted in the medial prefrontal cortex, nucleus accumbens, caudate-putamen, substantia nigra, and ventral tegmental area. Brain nuclei were quickly collected from hand-cut brain sections (about 500-μm thick) and homogenized in 0.25 M sucrose. Samples proceeded from at least four different litters per age and experimental treatment. Dopamine (DA), dihydroxyphenylacetic acid (DOPAC), and norepinephrine (NE) levels were analyzed using HPLC with electrochemical detection according to our previously published method.[13] Values were expressed as ng/mg of protein. Protein was determined by the method of Lowry *et al*.[14]

Statistics

For data analysis, normality of distribution was tested, and two-way analysis of variance (ANOVA, gender × treatment) or three-way ANOVA (age × gender × treatment) was applied as required, followed by an appropriate *post hoc* test (Student–Newman–Keuls).

RESULTS

In the present study, 26 litters were involved (14 saline control and 12 Meth-exposed litters). Neonatal Meth exposure resulted in a 4.3% mortality rate, whereas control litters had no mortality.

Places of Origin of Dopaminergic Cells

On PND7, catecholamine levels of the ventral mesencephalon of Meth-exposed and controls rats, males and females, were analyzed using two-way ANOVA (variables: treatment, gender, six to nine determinations per experimental group). No differences were found in NE, DA, or DOPAC levels or in the DOPAC/DA ratios.

On PND14 and PND30, NE levels in the substantia nigra were altered by Meth treatment ($F(1,54) = 9.30$, $P < .01$) and by age × treatment interaction ($F(1,54) = 4.10$, $P < .05$), as shown in FIGURE 1A. On PND14, Meth-exposed males had higher NE levels than control males ($P < .05$); on PND30, the effect was verified in males ($P < .05$) and females ($P < .05$). DA levels were not significantly affected, although treatment tended to increase them ($F(1,54) = 3.50$, $P = .0668$), in the same groups that presented NE variation (FIG. 1B). DOPAC levels varied only by the interaction

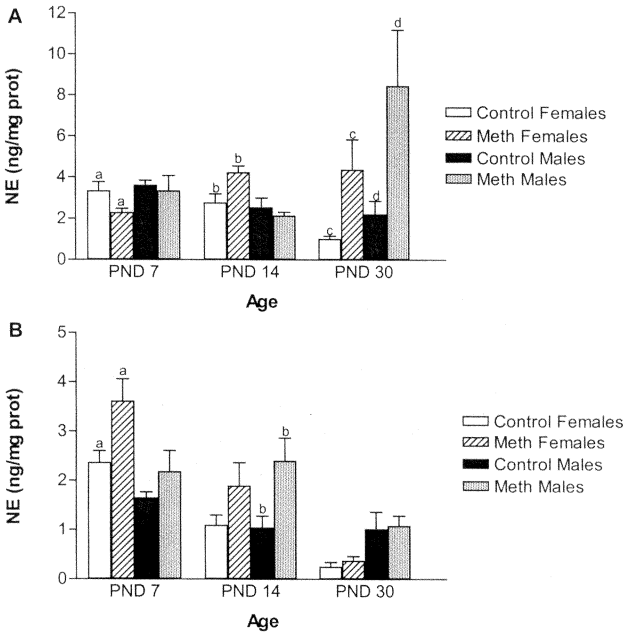

FIGURE 1. Effects of neonatal Meth exposure on neurochemical determinations in the substantia nigra of male and female PND14 and PND30 rats. Some of the rats were neonatally exposed to Meth; others constituted control groups. Each value is a mean ± SEM of six to nine determinations per group. Data were assessed by three-way ANOVA (age × gender × treatment) followed by the Student–Newman–Keuls test. Columns with the same letter (a, b, or c) are statistically different ($P < .05$). (**A**) Norepinephrine (NE) levels. (**B**) Dopamine (DA) levels. (**C**) DOPAC/DA ratio.

of age × gender ($F(1,51) = 8.24$, $P < .01$): males had higher DOPAC levels than females, but the difference was significant only on PND14 ($P < .05$). Meth effects upon catecholamine levels in the substantia nigra were also visible in the DOPAC/DA ratio. In this area, the DOPAC/DA ratio varied with treatment ($F(1,51) = 4.42$, $P < .05$), age ($F(1,51) = 7.88$, $P < .01$), and age × gender interaction ($F(1,51) = 18.90$, $P < .0001$) (FIG. 1C). The age effect was related to gender because the DOPAC/DA ratio was lower on PND30 compared with PND14, but only in males.

Gender effects were verified on PND14 and PND30: DOPAC/DA was higher in males than in females (PND14: controls, $P < .01$; Meth, $P < .005$; PND30: controls, $P < .05$; Meth, $P < .005$). The treatment effect was also related to gender. Meth-treated males had lower DOPAC/DA ratios than controls (PND14: tendential; PND30: $P < .05$).

In the ventral tegmental area, ANOVA only discriminated a gender effect over DOPAC levels ($F(1,40) = 4.61$, $P < .05$). Neonatal Meth exposure did not affect catecholamine levels in the ventral tegmental area of PND14 and PND30 rats.

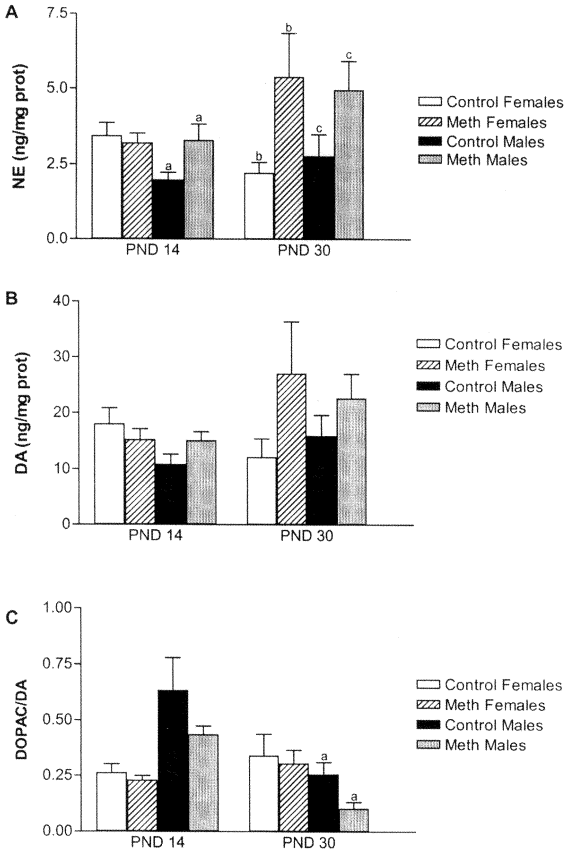

FIGURE 2. Effects of neonatal Meth exposure upon norepinephrine (NE) levels in the (**A**) nucleus accumbens and (**B**) caudate-putamen of male and female PND7, PND14, and PND30 rats. Some rats were neonatally exposed to Meth; others constituted control groups. Each value is a mean ± SEM of six to nine determinations per group, expressed as ng NE/mg protein. Data were assessed by three-way ANOVA (age × gender × treatment) followed by the Student–Newman–Keuls test. Columns with the same letter (a, b, c, or d) are statistically different ($P < .05$).

Sites of Termination of Dopaminergic Cells

In the caudate-putamen, NE levels varied with age ($F(2,80) = 30.64$, $P < .001$), treatment ($F(1,80) = 14.34$, $P < .001$), and with age × gender interaction ($F(1,80) = 8.43$, $P < .001$), as shown in FIGURE 2A. On PND30, control females had lower NE levels than control males ($P < .05$), whereas on PND7, controls evidenced the opposite variation ($P < .05$). Meth treatment increased NE levels on PND7 females ($P < .05$) and on PND14 males ($P < .01$) and females ($P \approx .06$); on PND30, this effect did not reach significant levels. DA levels were affected only by age ($F(2,79) = 15.20$,

$P < .001$) and by the interaction age × gender ($F(2,79) = 5.25$, $P < .01$). In control males, DA levels increased from PND14 to PND30 ($P < .05$), whereas in females the values did not change; on PND14, females had higher DA levels than males ($P < .05$). DOPAC levels varied only with age ($F(2,77) = 37.82$, $P < .001$), gender ($F(1,77) = 12.29$, $P < .001$), and age × gender interaction ($F(2,77) = 3.37$, $P < .03$). In control animals, DOPAC levels were higher in males than in females on PND14 ($P < .05$) and on PND30 ($P < .01$). In the caudate-putamen, the DOPAC/DA ratio did not vary with Meth or with the interactions of Meth and other variables.

In the medial prefrontal cortex, age influenced NE ($F(1,54) = 12.06$, $P < .002$) and DA levels ($F(1,54) = 10.48$, $P < .003$). DA increased with age (control groups: males, $P < .05$; females, $P < .05$), as did NE. This effect was related to gender (interaction age × gender: NE: $F(1,54) = 4.53$, $P < .05$; DA: $F(1,54) = 4.43$, $P < .05$). PND30 male controls had higher NE and DA levels than control females ($P < .05$ in both cases). Regarding DOPAC, there were no significant differences. Neonatal Meth exposure did not affect catecholamine levels in medial prefrontal cortex of PND14 and PND30 rats.

In the nucleus accumbens, NE levels were affected by Meth treatment ($F(1,71) = 6.45$, $P < .02$) and by the interactions of age × treatment ($F(2,71) = 7.06$, $P < .002$) and age × gender ($F(2,71) = 3.29$, $P < .05$), as shown in FIGURE 2B. On PND7, Meth-treated females had lower NE levels than controls ($P < .05$). Meth-treated animals had higher NE levels than respective controls, on PND30 (both in males, $P < .05$, and in females, $P < .05$) and on PND14 (only in females, $P < .05$). DA and DOPAC levels varied with age (DA: $F(2,74) = 10.43$, $P < .001$; DOPAC: $F(2,70) = 35.97$, $P < .001$) and with the interaction of age × gender (DA: $F(2,74) = 9.04$, $P < .001$; DOPAC: $F(1,70) = 6.21$, $P < .01$). In males, DA and DOPAC levels increased from PND14 to PND30 (DA: $P < .05$; DOPAC: $P < .05$). On PND30, DA and DOPAC levels in the nucleus accumbens were higher in males than in females (DA, $P < .05$; DOPAC, $P < .05$). In this brain area, DOPAC/DA ratio varied with age ($F(2,69) = 57.80$, $P < .001$), gender ($F(1,69) = 13.11$, $P < .001$), and the interaction age × gender ($F(1,69) = 9.01$, $P < .0005$). Age effect was related to gender (in males, it was lower on PND7 than on PND14, and it was highter on PND14 than on PND30). The gender effect was related to age, being evident only on PND7 and PND14: the DOPAC/DA ratio was lower in females than in males (control animals: PND7, $P < .05$; PND14, $P < .05$).

DISCUSSION

In the present work, Meth exposure was applied during the period of neonatal development of the dopaminergic system, using a sufficient (but not excessive) Meth dose (as demonstrated by the reduced mortality rate, 4%). This protocol is distinct from other experimental paradigms of neonatal Meth exposure by the sustained and prolonged period of exposure (starting on PND1), by the moderate Meth dosage, and by the ages selected for the assessment of effects (PND7, PND14, and PND30 in the first month of life). Male and female pups were assessed because it was previously suggested that males are more susceptible than females to Meth exposure, whether they are adults[15–17] or pre-adolescent.[18,19]

The present results agree with communications that neonatal Meth administration is associated with smaller neurochemical changes compared with the adult exposure

(for example, in the rat[20–22] and in the mouse[23]), which are also consistent with results on peri-adolescent rats exposed to different dopaminergic drugs.[24] In fact, in the dopaminergic brain areas studied, DA levels were only affected in one area (substantia nigra), and in an indirect manner, because effects were observed in the DOPAC/DA ratio, in PND30 Meth-exposed males. On PND30, sustained neonatal Meth exposure increased tyrosine hydroxylase activity in the caudate-putamen and substantia nigra and increased tyrosine hydroxylase mRNA levels in the substantia nigra of male pups.[19]

Sustained neonatal Meth exposure, however, altered NE levels in the substantia nigra, caudate-putamen, and nucleus accumbens. Although Meth is known to act upon the noradrenergic system,[2,25,26] these findings are unexpected; the areas evaluated in this study have low NE levels. In fact, the caudate-putamen presents very sparse noradrenergic fibers, and in the nucleus accumbens, noradrenergic fibers are present only in areas that merge with other telencephalic cell groups.[27] These fibers originate in the locus coeruleus, which sends wide projections to almost all major brain structures; however, in the mesencephalic tegmentum, only a few fibers were identified.[27] In the nucleus accumbens, on PND7, Meth-exposed females had lower NE levels than controls, whereas on PND30, the variation was the reverse: Meth-exposed pups had higher NE levels than respective controls, confirming the trend observed already on PND14 (only in females). In the caudate-putamen, NE levels were increased by Meth exposure in the end of the first week in females, and in the end of the second week in males. On PND30, Meth effects were no longer observed in this area, and it was reported that sustained Meth exposure increased striatal tyrosine hydroxylase activity in male rats at this age.[19] NE levels were also increased in the substantia nigra of Meth-exposed pups (PND14 females and PND30 male and female pups). In the substantia nigra, DA levels varied within the same pattern as NE, although in a non-significant manner. In this area, the decreased DOPAC/DA ratio after neonatal Meth exposure may result from the combined action of increased DA (perhaps due to increased synthesis of the neurotransmitter, increased release, and/or decreased recapture) and decreased DOPAC levels. The lack of significant differences in DA in the mesencephalon after neonatal Meth exposure is consistent with reports that used shorter periods of exposure and higher doses.[28]

The evidence of NE association with the response to neonatal Meth exposure, in the nucleus accumbens, caudate-putamen, and substantia nigra, is a relevant point of the present work. In adult rodents (rat and mouse), pretreatment with a noradrenergic neurotoxin [*N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4)] significantly enhanced Meth-induced nigrostriatal DA depletion[29,30]; it was proposed that NE might play a neuroprotective role in these rodent species.[29] The developing rat is more resistant to Meth-induced neurochemical changes than adults.[20–22,31] Perhaps the observed increase in NE levels associated with sustained neonatal Meth enhances the resistance to the effects on the dopaminergic system of the pre-adolescent rat. The increased NE levels were found on PND30 (after one month of daily Meth exposure) in the nucleus accumbens, an area associated with the phenomena underlying addiction and sensitization to drugs of abuse,[32,33] and in the substantia nigra, an area implicated in movement regulation[34] and addiction.[35]

Acute Meth administration was shown to alter the normal functioning of the locus coeruleus in the adult rat.[25] In consideration of the present results and since the sparse NE innervation of the brain areas herein studied originates in the locus coer-

uleus, it is suggested that this noradrenergic brain area should be further studied in the context of sustained neonatal Meth exposure. This may contribute to understanding the enhanced resistance of young mammals to the action of this psychostimulant.

CONCLUSIONS

Neonatal Meth exposure throughout the first month of life of the rat affected males more intensely than females, in terms of altering catecholamine levels in dopaminergic areas. In the substantia nigra, the DOPAC/DA ratio in males was decreased by neonatal Meth. In the ventral tegmental area and medial prefrontal cortex, catecholamine levels were not affected by neonatal Meth exposure on PND14 and PND30. Neonatal Meth increased NE levels in the caudate-putamen on PND7 and PND14, and in the substantia nigra and nucleus accumbens on PND14 and PND30.

The implications of increased NE levels associated with repeated neonatal Meth exposure in dopaminergic areas remain to be fully clarified, but may contribute to the described enhanced resistance of developing mammals to the effects of this psychostimulant, in comparison to adults.

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