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Research report

Mapping of the prenatal and postnatal methamphetamine effects on D_1-like dopamine, M_1 and M_2 muscarinic receptors in rat central nervous system

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ABSTRACT

Methamphetamine (MA) is worldwide known drug with high potential for addiction that causes dopamine, noradrenaline and serotonin release. MA is also able to increase acetylcholine levels in adult rodents. The aim of this study was to map changes in D_1-like dopamine receptors (DR), M_1 and M_2 muscarinic receptors (MR), and the total number of MR (M_1-M_5 MR) in the CNS of rats exposed to MA prenatally and in adulthood. Rat mothers were exposed to MA (5 mg/kg s.c.) or saline during the entire gestation period and their male offspring were administered in adulthood with single MA (1 mg/kg) or saline injection. Thus, the animals were divided into 4 groups: prenatally MA-exposed rats treated with saline (MA/S) or MA (MA/MA) in adulthood and prenatally saline-exposed rats treated with saline (S/S) or MA (S/MA) in adulthood. One hour after the acute treatment animals were sacrificed and their brains were removed. The numbers of M_1, M_2, total MR, and D_1-DR were measured by autoradiography. The main effect was detected in the hippocampus with the most affected M_1 MR. D_1-DR were decreased in motor cortex and substantia nigra. M_1MR were decreased in caudate-putamen, dorsal hippocampus, CA1, CA3 and dentate gyrus (DG). M_2MR were decreased in DG only. Total number of MR was moreover decreased in dorsal hippocampus, CA1, CA3 and DG. Our results have shown different patterns of changes in DR and MR, suggesting a pilot role of M_1 MR in the CNS changes induced by prenatal and adult MA exposure.

1. Introduction

Dopamine receptors are implicated in many neurological processes, including motivation, pleasure, learning and memory (Myslivecek et al., 1997), and fine motor control, as well as modulation of neuroendocrine signaling (Girault and Greengard, 2004).

According to their structural similarities, dopamine receptors are divided into the two groups (for review see (Benes et al., 2012; Emilien et al., 1999; Girault and Greengard, 2004)): D_1-like (D_1 and D_5 subtypes) and D_2-like (D_2, D_3 and D_4 subtypes). The families of dopamine receptors differ in the coupling to G proteins and subsequent steps of intracellular signalization. While D_1-like receptors activate adenylyl cyclase via G_s protein, D_2-like family (mainly pre-synaptic D_2 receptors) inhibits adenylyl cyclase via Gi protein activation. Moreover, coupling with $G_{q/11}$ protein allow D_2 receptor subtype to activate phospholipase C. In simplification, D_1-like receptors are localized post-synaptically, D_2-like receptors pre-synaptically (Sato et al., 2014).

Muscarinic receptors (MR) can be divided into odd-numbered (M_1, M_3, M_5) and even-numbered (M_2, M_4) subtypes (Brown, 2010). While odd-numbered subtypes are coupled to $G_{q/11}$ proteins and thus activate phospholipase C-proteinkinase C pathway, even-numbered subtypes are able to inhibit adenylyl cyclase (and proteinkinase A) via G_i protein (Benes et al., 2013). Also, it is possible to generalize, that odd-numbered subtypes (Brown, 2010) are connected with membrane depolarization, increased excitability while even-numbered cause presynaptic inhibition. In general, all subtypes are widely distributed in the central nervous system, but in some brain areas some of them are less expressed than the others (for MR subtype distribution see (Eglen, 2005)).

When studying the receptor density, it is important to emphasize that the total number of receptors need not to be changed whilst M_1 MR or M_2 MR are. This can be caused, for example, by decrease in M_1 MR and parallel increase in other subtypes (M_2, M_3, M_4, M_5) what would make total number of MR unchanged.

Methamphetamine (MA) is worldwide known drug with high potential for addiction that is also favorite drug for drug-addicted pregnant women because of its psychostimulant and anorectic effects

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(Marwick, 2000). While MA is able to pass thanks to its lipophilic nature through hematoencephalic barrier, the placental barrier is easy obstacle (Rambousek et al., 2014a). Its methyl group (making it more lipophilic) also increases the resistance against enzymatic degradation by monoaminooxidase (MAO).

Our previous behavioral studies demonstrated that prenatal MA exposure impairs postnatal development of rat pups (Slamberova et al., 2006), cognitive functions in adulthood (Macuchova et al., 2017; Slamberova et al., 2005), affects anxiety and social behavior (Macuchova et al., 2016; Slamberova et al., 2015) pain sensitivity (Yamamotova et al., 2011) and seizure threshold of adult male rats (Bernaskova et al., 2011; Bernášková and Šlamberová, 2017; Slamberova and Rokyta, 2005). Further, we demonstrated that rats exposed to MA *in utero* have shown changes in the mesolimbic dopaminergic system and are more sensitive to the administration of the acute dose of MA (1 mg/kg) in adulthood, which correspond with the increased locomotion (Bubenikova-Valesova et al., 2009; Fujakova-Lipski et al., 2017). Furthermore, we found that the metabolites of dopamine, such as dihydroxyphenylacetic acid and homovanillic acid, are also changed in prenatally MA-exposed rats (Bubenikova-Valesova et al., 2009).

The mechanism of action of MA is multiple. MA cause dopamine, noradrenaline and serotonin release what consequently increase the post-synaptic receptor stimulation (Thrash et al., 2009).

Dopamine is critically involved in drug addiction processes. Changes in the expression of dopamine receptors are documented after administration of addictive drugs. Preferentially, dopamine-1 receptor (D1R) is involved in the locomotor effects and in the rewarding/reinforcing effects of MA (Ramos et al., 2004). After chronic administration of MA decreased expression or binding to dopamine-2 receptor (D2R) were observed (Kim and Han, 2009; Segal et al., 2005). The expression of D2R was fully recovered after 30 days of abstinence. Dopamine-3 receptor (D3R) is associated with sensitization to MA (Jones et al., 2007) and after administration of MA these receptors are down-regulated. It has been shown that D4 receptors are not influenced by administration of MA and D5 receptors are difficult to distinguish from D1R (Le Foll et al., 2009). Taken together, dopamine receptors play a key role in MA addiction.

The changes in neurotransmitter levels could be the basis for receptor changes. Thus, MA administration (postnatally, 7 days, 10 mg/kg, i.p.) was able to decrease D_1 DR (in fact it was D_1-like receptors what is not emphasized by authors) in murine striatum (Park et al., 2011).

MA is also able to increase acetylcholine levels in adult mice (Siegel et al., 2010) after long-term exposure (5 mg/kg once a day from postnatal day 11–20).

The first paper studying postnatal MA effects (repeated exposure, five doses in six-hour interval between doses) on MR was the study of (McCabe et al., 1987) that showed no change in M_1 MR in any of the brain regions examined following MA treatment and decrease in ^{3}H-NMS binding (what indicates decrease in total number of MR). In another study (Lee et al., 2008), the authors showed increased M_1 MR mRNA after repeated MA treatment in the dentate gyrus and in the frontal cortex and the CA2 region of the hippocampus after acute and repeated treatment (one dose 0.1 ml/10 g, seven doses (one per day)) in male adult mice.

Long-term exposure (Siegel et al., 2010) increased the number of M_1 MR in the hippocampus, not in cortex (measured by direct membrane binding). M_2 MR were not affected in this study.

Based on the above the aim of the present study was to investigate the effects of prenatal MA on total number of MR, M_1 and M_2 MR using autoradiography in the brain of animals postnatally exposed to single dose of MA. Brain structures that may be associated with drug addiction (mesolimbic system), cognition (hippocampus) and other behaviors that are affected with prenatal or acute MA exposure as shown in our previous studies were analyzed.

2. Experimental procedures

The procedures for animal experimentation in this study were reviewed and approved by the Institutional Animal Care and Use Committee and were in agreement with the Czech Government Requirements under the Policy of Human Care of Laboratory Animals (No. 246/1992) and with subsequent regulations from the Ministry of Agriculture of the Czech Republic.

2.1. Drugs

D-methamphetamine hydrochloride, physiological saline, (+)-Butaclamol hydrochloride, atropine sulfate were purchased from Sigma-Aldrich. Pirenzepine [*N*-methyl-^{3}H] (83.4 Ci/mmol) was from American Radiolabeled Chemicals (ARC, Inc.). AFDX 384 [2,3-dipropylamino-^{3}H] (106.5 Ci/mmol), quinuclidinyl benzilate L-[benzilic-4,4′-^{3}H] (46 Ci/mmol), SCH 23390 [*N*-methyl-^{3}H] (85 Ci/mmol) were from Perkin Elmer NEN.

2.2. Animals

2.2.1. Drug administration

Adult female and male albino Wistar rats (375–400 g) provided by Charles River Laboratories International, Inc. were delivered by AnLab (Prague, the Czech Republic). Animals were housed four per cage by sex and left undisturbed for a week in a temperature-controlled (22°–24 °C) colony room with free access to food and water on a 12 h (light):12 h (dark) cycle with lights on at 06:00 h. Females were impregnated as described in our previous study (Šlamberová et al., 2005). In total, 24 dams were randomly assigned to either the MA-treated or saline-treated group. On gestational day (GD) 1 the daily injections of MA or saline started and continued until the day of delivery, which usually occurred on GD 22. MA was diluted in distilled water in concentration of 5 mg/ml and injected subcutaneously (s.c.) in a volume of 1 ml/kg; saline was injected s.c. at the same time and volume as MA. The dose 5 mg/kg of MA was chosen because it induces similar fetal brain drug concentrations and similar behavioral changes to those found in humans (Schilling et al., 1996).

The day of the delivery was indexed as postnatal day (PD) 0. On PD 1, pups were weighed, tattooed for identification, and cross-fostered (for detailed information see (Hrubá et al., 2009)). Whenever possible, the number of male and female pups raised by a dam was equal. On PD 21, pups were weaned and group-housed by sex. Animals were left undisturbed until adulthood.

Only male offspring (PD 80–90) were used in the present study. 20 adult males, 10 prenatally exposed to MA and 10 prenatally exposed to saline, were assigned to acute saline and acute MA treatment. Single injection of saline (1 ml/kg) or MA (1 mg/ml/kg) was s.c. administered 1 h prior to decapitation. The dose of MA was chosen based on our previous studies (Šlamberová et al., 2010) showing that this dose does not induce stereotypical behaviour. The timing of the drug application was also chosen based on our previous study (Rambousek et al., 2014b) that showed that peak MA level in the brain (not in the blood) occurred between the 45th and 60th minute after administration.

Thus, based on prenatal drug exposure and the acute adult treatment, the animals were divided to 4 experimental groups: Prenatally MA-exposed rats treated with saline (MA/S) or MA (MA/MA) in adulthood and prenatally saline-exposed rats treated with saline (S/S) or MA (S/MA) in adulthood.

2.2.2. Brain structures

Rats were sacrificed by decapitation. Brains were rapidly removed from the scull, frozen in dry ice and stored at −80 °C until cryosectioning. After preparation for autoradiography, following brain areas were analyzed: motor cortex (Cx), caudate-putamen (CPu), substantia nigra (SN) ncl. Accumbens (NAc), olphactory tubercle (OT),