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Methamphetamine-induced expression of *zif268* mRNA is prevented by haloperidol in mice lacking μ -opioid receptor

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Abstract

We investigated the role of μ -opioid receptor (μ -OR) and dopamine receptor in the modulation of methamphetamine (METH)-induced expression of *zif268* mRNA in the striatum of mice. Four groups of wild-type and μ -OR knockout mice were given a single daily intraperitoneal injection of saline (control; group 1) or METH (10 mg/kg; groups 2–4) for 7 consecutive days. On day 11 (after 4 abstinent days), groups 1 and 2 were challenged with saline, group 3 was challenged with METH (10 mg/kg), and group 4 was challenged with dopamine receptor antagonist haloperidol (0.06 mg/kg, subcutaneous injection) plus METH (10 mg/kg). Two hours after the last saline or METH injection, mouse brain tissues were taken for *zif268* mRNA analysis using *in situ* hybridization histochemistry. In comparison to corresponding saline control group (group 1), striatal *zif268* mRNA levels were unchanged in group 2 and increased in group 3 in both wild-type and μ -OR knockout mice and without genotype difference. METH challenge-enhanced expression of *zif268* mRNA was completely abolished by pre-administration of haloperidol (group 4) in μ -OR knockout mice but not in wild-type mice. The results suggest a crosstalk of the two neurotransmitter systems in modulation of METH-induced IEG expression, because only in μ -OR knockout mice in which dopamine receptors were blocked were METH-induced *zif268* expression abolished. METH-induced *zif268* expression was not altered in μ -OR knockout mice without blockade of dopamine receptors or wild-type mice with blockade of dopamine receptors.

Keywords

methamphetamine; *zif268* mRNA; *in situ* hybridization; μ -OR knockout mice; haloperidol

Introduction

Methamphetamine (METH) is a psychostimulant that activates several neurotransmitter systems (Miller and O'Callaghan, 1994; Shaw, 1999). As a member of the amphetamine

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family, METH has much more potent and longer lasting central effects than amphetamine. Chronic exposure to amphetamine or METH not only leads to addiction but also produces significant neurotoxicity in humans (Volkow et al., 2001) and experimental animals (Seiden and Sabol, 1996; Kaewsuk et al., 2009). METH-induced neurotoxicity is characterized by a persistent depletion of dopamine in the dopaminergic system and long-lasting behavioral abnormalities (Kita et al., 2000; Jeng et al., 2005). Recently, we found that repeated administration of METH decreases the striatal intensity of tyrosine hydroxylase (the rate-limiting enzyme of dopamine synthesis and the target for many neurotoxicants and drugs) in wild-type mice but not in the μ -OR knockout mice (unpublished data). Also, we observed that METH produces hyperlocomotor activity at a low dose (0.62 mg/kg) and stereotyped behaviors at high doses (2.5 and 10 mg/kg). Repeated administration of METH led to progressively enhanced and persistent locomotor and stereotyped behaviors, called behavioral sensitization. The μ -OR knockout mice were less sensitive than wild-type mice to METH-induced hyperlocomotor activity and stereotyped behaviors. Contrarily, the μ -OR knockout mice showed approximately 3-fold more sensitivity to the dopamine receptor antagonist haloperidol in counteracting METH-induced stereotyped behaviors (Shen et al., 2010). It remains unclear why there are genotype differences regarding METH-induced depletion of striatal tyrosine hydroxylase and behavioral responses.

Immediate early genes (IEGs) are actively, transiently and rapidly responsive to a wide variety of cellular stimuli (Davis et al., 2003). It has been reported that IEGs play a role in the transmission of information from cell surface receptors to the genetic material in many instances of neuronal plasticity, including development of seizure susceptibility, long-term potentiation and drug-induced behavioral changes (Robertson, 1992). Thus, studies on the inductions of IEGs may provide a useful tool in understanding the mechanisms and functions of transsynaptic activation of certain central neuronal pathways.

Changes in expression of IEGs induced by drugs of abuse have been proposed to be associated with specific behavioral changes (Harlan and Garcia, 1998; Shilling et al., 2006). Several lines of evidence suggest that METH and morphine induce the expression of IEGs in the brains of rodents. For example, acute METH enhances expression of *c-fos* and *zif268* mRNA in the frontal cortex and striatum of mice (Hirata et al., 1998) as well as *arc* mRNA in the striatum and cortex of rats (Yamagata et al., 2000). Intra-striatum injection of *c-fos* antisense blocks METH-induced ambulatory locomotor activity in mice (Umekage et al., 1997). Morphine increases *c-fos* mRNA expression in the striatum of rats and this induction is completely abolished by a non-selective opioid receptor antagonist naloxone (Chang et al., 1988). Moreover, Horner and Keefe (2006) found that μ -OR antagonist clocinnamox blocks METH-induced expression of *zif268* mRNA in the striatum of rats. Morphine is a μ -OR agonist whereas METH is an indirect dopamine receptor agonist. These data indicate that both μ -OR and dopamine receptor are implicated in modulation of the expression of IEGs.

This study is designed to determine the role of μ -OR and dopamine receptor in METH-induced expression of *zif268* by using μ -OR knockout mice and dopamine receptor antagonist haloperidol. *Zif268* is a mammalian transcription factor that is also called Egr1 (early growth response protein 1). It is known that *zif268* is required for long-lasting behavioral effects of drugs of abuse (Valjent et al., 2006; EL Rawas et al., 2009). In the study, we tested the hypothesis that both of the two neurotransmitter receptors are involved in modulation of METH-induced expression of *zif268* mRNA. The μ -OR knockout mice are less sensitive to METH in enhancing expression of *zif268* but more sensitive to dopamine receptor antagonist in counteracting METH-induced *zif268* expression.

Materials and methods

Chemicals

Deoxyadenosine 5'-(α -thio) triphosphate, [35 S]-ATP (specific activity: 1250 Ci/mmol) were purchased from PerkinElmer Life Science (Boston, MA, USA). METH and other chemicals were purchased from Sigma (St. Louis, MO, USA).

Animals

The μ -OR knockout mice used in this study were developed by Loh et al. (1998) and maintained on a 1:1 hybrid genetic background (C57/BL6 and 129/Ola), as described. Mice were maintained in an animal room on a 12-h light/dark cycle and at constant temperature ($22 \pm 2^\circ$ C). All procedures for animal care and breeding were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Mississippi Medical Center Animal Care and Use Committee.

Experimental protocol and sample preparations

Male wild-type and μ -OR knockout mice, ranging from 8 to 12 weeks old (body weight 20–25 g) were used in this study. Mice from each genotype were randomly divided into 4 groups with 8 mice each group. METH was freshly dissolved in saline before use and injected at a volume of 10 ml/kg of the body weight. For initiation of behavioral sensitization, mice were daily intraperitoneally (*i.p.*) injected with saline (control; group 1) or METH (10 mg/kg; group 2–4) for 7 consecutive days. On day 11, each group of mice was subcutaneously (*s.c.*) injected with saline (groups 1–3) or haloperidol (0.06 mg/kg; group 4) and then, 30 min later, injected with saline (groups 1 and 2) or METH (10 mg/kg, *i.p.*; groups 3 and 4). In the previous behavioral study, we found that acute administration of METH to mice produced significantly hyperlocomotor activity at low doses and stereotyped behavior at high doses (2.5 mg/kg and above). After repeated administration of METH, wild-type mice showed progressively enhanced locomotor activity (at 0.62 mg/kg) and stereotyped behaviors (at 2.5 and 10 mg/kg), as observed on experimental days 4 and 7, which suggests the development of behavioral sensitization (reverse tolerance) to the psychostimulant in these animals. METH at doses of 2.5 mg/kg and below did not induce behavioral sensitization but 10 mg/kg of METH produced a slight behavioral sensitization, as observed on day 7, in the μ -OR knockout mice (Shen et al., 2010). Behavioral sensitization is well known to be a long-lasting behavioral change. Animals remain sensitized for weeks or months after prolonged periods of abstinence (Chiu et al., 2005; Robinson and Berridge, 2008). Therefore, 10 mg/kg of METH and a 7-day METH initiation plus 4 abstinent day treatment protocol were used in this study.

Two hours following the last injection, mice were sacrificed by decapitation. The brains were removed from the skull and immediately frozen in liquid nitrogen. Coronal sections of 14 μ m thickness were cut in a microtome cryostat (Cryo 2000, Tissue-Tek) at -20° C. The sections were thaw-mounted on gelatin-coated slides and stored at -80° C until used.

Preparation of probes for *in situ* hybridization

The oligonucleotide probe (Invitrogen Corporation, CA, USA) was complementary to mRNAs encoding mouse *zif268*. The sequence for *zif268* was 5'-GCG GCG AAT CGC GGC GGC TCC CCA AGT TCT GCG CGC TGG GAT CTC-3' (Economou et al., 2004). Oligonucleotide (10 pmol) was labeled at 3' end with 5 μ l of 35 S-dATP using 35 U of terminal deoxynucleotidyltransferase (PerkinElmer Life Science 3' end oligonucleotide labeling system) for 60 min at 37° C. The labeled probes were purified utilizing Centri-Spin columns (Princeton separation, NJ, USA) centrifuged at 3,000 rpm for 2 min. Two μ l of this solution were counted by a liquid scintillation counter to assess the efficiency of labeling.

Hybridization

The slide-mounted sections were air-dried and fixed in 4% paraformaldehyde in 0.1 M phosphate-saline buffer for 15 min at 4°C, then rinsed with 0.1 M phosphate-saline buffer for 3 min at room temperature. Brain sections on the slides were immersed in 0.1 M triethanolamine-HCl and acetic acid for 10 min at 4°C. The sections were washed with 0.1 M phosphate-saline buffer for 3 min at room temperature, then dehydrated in a series of ascending concentration of ethanol (70 and 100% for 5 min each). The dehydrated sections were incubated with the hybridization mixture, which contained 50% formamide, 4 × SSC (1 × SSC = 0.15 M NaCl and 0.015 M sodium citrate), 10% Dextran sulfate, 5 × Denhardt's, 0.25 mg/ml tRNA, 0.5 mg/ml ssDNA, 100 mM dithiothreitol, and 1 × 10⁶ cpm/slide of ³⁵S-labeled oligonucleotide. Slides were covered with hybridization coverslips and incubated overnight at 38°C in a humid chamber. After hybridization, the coverslips were floated off in 1 × SSC at room temperature. Then slides were washed in 1 × SSC for 15 min at 55°C twice and 0.5 × SSC for 15 min at 55°C twice, plus final wash in 0.5 × SSC for 10 min at room temperature twice. Slides were rinsed in distilled water, dehydrated in 70 and 100% ethanol for 5 min at room temperature each and immediately air-dried. Autoradiograms were obtained by juxtaposition of the tissue sections with Kodak BioMax MR Film (Eastman Kodak Co., Rochester, NY, USA) for 2 weeks at room temperature. The films were developed in Kodak D19 (Eastman Kodak Co., Rochester, NY, USA) and fixed.

Statistical analysis

The autoradiograms were analyzed using a scanning densitometer (Personal Densitometer, Molecular Dynamics, Sunnyvale, CA, USA), operating on the image acquisition and analysis program Image Quant 3.3 (Molecular Dynamics). Data were expressed as mean ± standard errors of the mean (S.E.M.). Each treated group was separately compared to that of the respective saline treated control. At the same treatment, μ-OR knockout mice were compared with that of the wild type controls. Statistical analysis was done by one-way ANOVA followed by a post-hoc Student-Newman-Keuls multiple comparison test. A difference was considered significant at P < 0.05.

Results

In situ autoradiograms and quantitative data of expression of *zif268* mRNA in the striatum from the wild type and μ-OR knockout mice are shown in Fig. 1 and table 1.

There was no significant difference in expression of *zif268* mRNA in all experimental groups except group 4 between wild type and μ-OR knockout mice. This result indicates that only lacking μ-OR in mice had no effect on METH-induced expression of *zif268* mRNA.

No significant change in expression of *zif268* mRNA was observed in 7 daily injections of METH followed by a 4-day drug free period in both wild type and μ-OR knockout mice (group 2, Table 1) when they were compared with that of the saline treated group (group 1, Table 1). However, there was a significant increase in *zif268* mRNA expression in both genotypes of mice that were challenged with METH following 4 drug abstinent days (group 3, Table 1). These results reveal that METH-induced expression of *zif268* mRNA is likely involved in early stages of target gene regulation in response to its exposure.

Haloperidol pretreatment 30 min before the challenge with METH on experimental day 11 completely blocked METH challenge-induced expression of *zif268* mRNA in μ-OR knockout mice, but not in wild type controls (group 4, Table 1).

Discussion

In the present study, no genotype difference in expression of *zif268* mRNA in the striatum was observed under basal (without METH treatment; group 1), METH-sensitized (group 2), and METH-sensitized with METH challenge conditions (group 3). The result indicated that lack of μ -OR in mice does not alter the magnitude of METH-induced expression of *zif268* mRNA in the brains, although a previous study showed that single injection of the μ -OR antagonist, clocinnamox, blocked METH-induced expression of *zif268* mRNA in the striatum of rats (Horner and Keefe, 2006). The difference results from the two studies may be due to the difference METH treatment protocols used or that there are certain compensatory changes in central neurotransmitter systems in μ -OR knockout mice since we noticed a higher dopamine receptor level in the striatum of naïve μ -OR knockout mice (Tien et al., 2003).

Recently, we found that METH produced hyperlocomotor activity at a low dose (0.62 mg/kg) and stereotyped behaviors at high doses (2.5 and 10 mg/kg). METH-induced behavioral responses were significantly attenuated in the μ -OR knockout mice in comparison to wild-type mice (Shen et al., 2010). Also, we noticed that repeated administration of METH (2.5 and 10 mg/kg) decreases the striatal intensity of tyrosine hydroxylase in wild-type mice but not in the μ -OR knockout mice (unpublished data). It has been proposed that the enhanced expression of IEGs is mediated with psychostimulant-produced behavioral abnormalities (Harlan and Garcia, 1998; Shilling et al., 2006). For instance, microinjection of *c-fos* antisense in the striatum blocks METH-induced ambulatory locomotor activity in mice (Umekage et al., 1997). Obviously, the present results do not support the idea that the difference of METH-produced neurotoxicity and behavioral abnormalities between wild-type and μ -OR knockout mice are related to the changes of IEGs in these animals.

However, the present study reveals the genotype difference of haloperidol counteracting METH-enhanced expression of *zif268* mRNA. Pre-administration of haloperidol completely eliminated METH challenge-induced expression of *zif268* mRNA in the μ -OR knockout mice but not in wild type controls (group 4, Table 1). Evidence indicates that haloperidol (1 mg/kg) enhanced expression of *zif268* mRNA in the striatum of rats that peaked 30–45 min after injection and returned to baseline within 2 hours (Nguyen et al., 1992). METH (4 mg/kg)-induced expression of *zif268* mRNA in the striatum of rats shows similar results, peaking at 45 min and returning to baseline within 3 hours after injection (Wang and McGinty, 1995). In the present study, we collected the brain tissue for determining *zif268* mRNA at 2.5 hours after the injection of haloperidol and 2 hours after METH administration. Thus, results from this study may represent an antagonistic effect of haloperidol on METH challenge-induced expression of *zif268* mRNA in METH-sensitized mice. The μ -OR knockout mice are more sensitive to haloperidol than wild type controls in counteracting METH challenge-induced expression of *zif268* mRNA. The change is consistent with our behavioral study results, in which haloperidol produced a more potent effect in counteracting METH-induced stereotyped behaviors in μ -OR knockout mice than that in wild-type controls (Shen et al., 2010).

In a previous study, we treated mice with different doses of METH for 7 consecutive days and sacrificed them on day 11 (4 days after last injection) for neurochemical measurements. METH at doses of 0.62 and 2.5 mg/kg produced significant decrease in D1 and D2 dopamine receptor ligand binding in the striatum and nucleus accumbens in the μ -OR knockout mice but not in wild-type mice. METH at a dose of 10 mg/kg also decreased D1 receptor ligand binding in these brain regions in the μ -OR knockout mice but not in wild-type mice, and increased D2 receptor ligand binding in both genotypes of mice (Tien et al., 2007). The results suggest that the METH-induced decrease in D1 dopamine receptor in the μ -OR knockout mice may be a characteristic change. Haloperidol is a D1/D2 receptor antagonist with relatively higher affinity for D2 sites. Because repeated METH exposure decreases D1 (probably D2 as well) receptor

levels in the brains of μ -OR knockout mice, a higher dopamine receptor occupancy by haloperidol in the brains of μ -OR knockout mice would be expected, which may explain a more potent efficacy of the antagonist counteracting METH-induced IEG expression and stereotyped behaviors in these mice. This possibility as well as how dopamine and μ -opioid receptor-mediated signal transduction cascades modulate METH-induced IEG expression, and thus leads to behavioral changes, need to be verified by further study.

Conclusion

Expression of *zif268* mRNA was not altered in μ -OR knockout mice without blockade of dopamine receptors or wild-type mice with blockade of dopamine receptors. However, METH-induced *zif268* expression was abolished in μ -OR knockout mice in which dopamine receptors were blocked. The results suggest a crosstalk of μ -opioid system and dopamine system in modulation of expression of *zif268* induced by METH.

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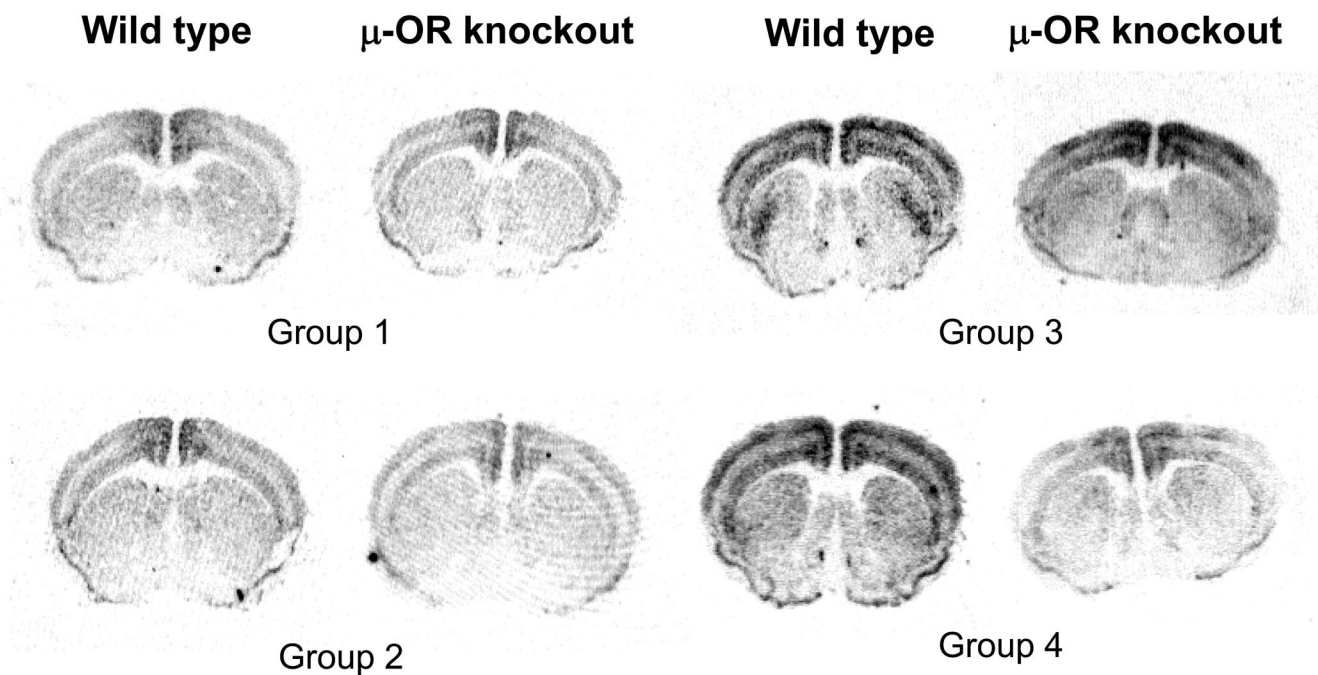


Figure 1.

In situ film autoradiograms showing the expression of *zif268* mRNA in the brain slides from the wild type and μ -OR knockout mice. Mice were daily intraperitoneally (*i.p.*) injected with saline (control; group 1) or METH (10 mg/kg; group 2–4) for 7 consecutive days. On day 11, each group of mice was subcutaneously (*s.c.*) injected with saline (groups 1–3) or haloperidol (0.06 mg/kg; group 4) and then, 30 min later, injected with saline (groups 1 and 2) or METH (10 mg/kg, *i.p.*; groups 3 and 4). Two hours following the last injection, mice were sacrificed by decapitation. The brain tissues were reserved for preparation of brain sections and *in situ* hybridization analysis.