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Role of μ-Opioid Receptor in Modulation of Preproenkephalin mRNA Expression and Opioid and Dopamine Receptor Binding in Methamphetamine-Sensitized Mice

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Abstract

We examined mRNA expression of preproenkephalin (PPE), a precursor of the endogenous opioid peptide enkephalin, and ligand binding to opioid and dopamine receptors in the striatum and nucleus accumbens in methamphetamine (METH)-sensitized μ-opioid receptor (μ-OR) knockout mice and their wild-type controls. Animals received daily intraperitoneal (i.p.) injections of METH (0, 0.625, 2.5, or 10 mg/kg) for 7 consecutive days to induce sensitization. Brain tissues were taken for biochemical analysis on experimental day 11 (4 days after the last injection). Expression of PPE mRNA and ligand binding were determined by in situ hybridization and autoradiography, respectively. Results indicate that there is an increase in PPE mRNA expression and a decrease in μ-OR ligand binding in METH-sensitized wild-type mice. These changes were not detected in METH-sensitized μ-OR knockout mice. A significant increase in δ-opioid receptor (δ-OR) ligand binding was found in μ-OR knockout mice. After repeated METH exposure, striatal and nucleus accumbal dopamine D1 receptor binding was decreased in μ-OR knockout mice but was not changed in wild-type mice. D2 receptor ligand binding was increased in wild-type mice and exhibited a biphasic change, with a decrease at 0.625 and 2.5 mg/kg doses of METH and an increase with 10 mg/kg of METH, in μ-OR knockout mice. These findings suggest that the μ-OR is involved in the regulation of METH-induced changes in an endogenous opioid peptide and dopamine receptors.

Keywords

autoradiography; in situ hybridization; psychostimulants; METH-induced sensitization; preproenkephalin

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Repeated administration of psychostimulants such as methamphetamine (METH) and amphetamine has been known to produce a progressively enhanced and persistent behavioral response in rodents, a phenomenon called "behavioral sensitization" (Robinson and Becker, 1986). Behavioral sensitization is considered to be related to compulsive drug-seeking behavior (Itzhak and Ali, 2002). The mesolimbic dopamine system in the central nervous system plays a critical role in the development of behavioral sensitization and is strongly modulated by nondopaminergic systems, including opioidergic, cholinergic, and γ-amino-

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butyric acid (GABA)-ergic systems. Previous studies have indicated that, once sensitization develops, a challenge dose of METH elicits behavioral hyperactivity, characterized by an increase in locomotor activity and stereotyped behaviors in mice (Phillips et al., 1994; Chiu et al, 2005). Our recent study demonstrated that μ-opioid receptor (μ-OR) knockout mice exhibited a diminished behavioral sensitization response to METH compared with wild-type controls (Shen et al., 2005). These results potentially implicate opioid receptors in the development of behavioral sensitization to METH.

The opioid system consists of various endogenous peptides and their receptors, classified into three main subtypes: μ-, δ-, and κ-opioid (Knapp et al., 1995; Satoh and Minami, 1995; Dhawan et al., 1996). It is well known that opioid peptide precursor genes preproenkephalin (PPE) and preprodynorphin (PPD) encode enkephalin and dynorphin, respectively. Enkephalin is an agonist at the μ- and δ-OR, and dynorphin is an agonist at the κ-OR. The striatum and nucleus accumbens are rich not only in dopamine receptors but also in opioid peptides and opioid receptors. Evidence indicates that the dopaminergic system regulates the expression of mRNA for PPE in the striatum (Tang et al., 1983; Mocchetti et al., 1987). For instance, chronic treatment (2–3 weeks) with the relatively selective D2 dopamine receptor antagonist haloperidol or the D1 dopamine receptor antagonist SCH 23390 increases the expression of mRNA for PPE in the rat striatum (Tang et al, 1983; Mocchetti et al, 1987). Pharmacological evidence also indicates that psychostimulants produce significant effects on the expression of mRNA for opioid peptides in these brain areas. For example, acute administration of METH enhances expression of striatal PPE mRNA and expression of striatal and accumbal PPD mRNA in the rat (Wang and McGinty, 1996; Horner et al., 2005). However, less attention has been focused on the effects of repeated treatments with METH on the expression of these opioid peptide mRNAs in the brain. In the present study, we examined central PPE mRNA expression and opioid and dopamine receptors in METH-sensitized μ-OR knockout mice in comparison with METH-sensitized wild-type control mice.

MATERIALS AND METHODS

Chemicals

[^{3}H]DAMGO (specific activity: 50 Ci/mmol), [^{3}H] DPDPE (specific activity: 45 Ci/mmol), [^{3}H]SCH23390 (specific activity: 86 Ci/mmol), [^{3}H]spiperone (specific activity: 15 Ci/mmol), and [^{35}S]ATP (specific activity: 1,250 Ci/mmol) were purchased from New England Nuclear (Boston, MA). METH and other chemicals were purchased from Sigma (St. Louis, MO).

Animals

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-hr light/dark cycle and at constant temperature (22°C ± 2°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 were used in this study. Mice were randomly divided into groups of eight mice each. Our previous study indicated that mice receiving seven daily injections with 2.5 mg/kg of METH show behavioral sensitization after challenge with 0.31–1.25 mg/kg of drug on day 11 (Chiu et al., 2005). In the present study, we have followed our previous procedure of drug administration

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(Chiu et al., 2005) but without drug challenge on day 11. Mice were injected (i.p.) with a single daily dose of 0.625, 2.5, or 10 mg/kg METH in the light cycle for 7 consecutive days, and the control animals received an equivalent volume of saline (10 ml/kg of body weight). Four days after the final injection (day 11), mice were sacrificed by decapitation, and the brains were removed from the skull and immediately frozen in liquid nitrogen. Coronal sections 20 μm thick were cut in a microtome cryostat (Cyro 2000; Tissue-Tek) at −20°C. The sections were thaw-mounted on gelatin-coated slides and stored at −80°C until use.

Preparation of Probes for In Situ Hybridization

The oligonucleotide probes (Invitrogen, Carlsbad, CA) were complementary to mRNAs encoding mouse PPE. The sequence for PPE was 5′-AAT TGA TGT CGC CTG GGC GAA CCA GGC GGT AGC TGC ATT TAG CGC AGT-3′ (Jamensky and Gianoulakis, 1999). Oligonucleotide (10 pmol) was labeled at the 3′ end with 5 μl [^{35}S]dATP using 35 U terminal deoxynucleotidyltransferase (NEN Life Science 3′ end oligonucleotide labeling system) for 60 min at 37°C. The labeled probes were purified by utilizing Centri-Spin columns (Princeton Separation, Princeton, NJ) and centrifuged at 3,000 rpm for 2 min.

Hybridization

The slide-mounted sections were air dried and fixed in 4% paraformaldehyde in 0.1 M phosphate-saline buffer (PBS) for 15 min at 4°C, then rinsed with 0.1 M PBS for 3 min at room temperature. Brain sections were immersed in 0.1 M tri-ethanolamine-HCl and acetic acid for 10 min at 4°C. The sections were washed with 0.1 M PBS for 3 min at room temperature, then dehydrated in a series of ascending concentrations 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 1×10^6 cpm/slide of [^{35}S]-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 twice in 1× SSC for 15 min at 55°C and twice in 0.5× SSC for 15 min at 55°C, with two final washes in 0.5× SSC for 10 min at room temperature. Slides were rinsed in distilled water, dehydrated in 70% and 100% ethanol for 5 min at room temperature, and immediately air dried. The labeled and dried slides containing calibration standards from brain paste of known radioactivity were apposed to Kodak BioMax MR film for 1–2 weeks at room temperature. The films were developed in Kodak D19 and fixed. The autoradiograms were analyzed by using a scanning densitometer (Personal Densitometer; Molecular Dynamics, Sunnyvale, CA), operating under the image acquisition and analysis program Image Quant 3.3 (Molecular Dynamics).

Autoradiography for μ- and δ-OR

OR densities were measured by quantitative ligand binding autoradiography according to Kitchen et al. (1997), with modifications. Briefly, the brain sections were preincubated at 4°C for 15 min in 50 mM Tris-HCl buffer (pH 7.4) containing 100 mM NaCl and 50 μM NaGTP and then incubated for 60 min in the same buffer with 5 nM [^{3}H]DAMGO or 15 nM [^{3}H]DPDPE at room temperature. The brain sections were incubated with 1 μM DAMGO or 1 μM DPDPE for nonspecific binding of μ- and δ-OR, respectively (Fan et al., 2002). The slides were placed in X-ray cassettes with calibration standards and juxtaposed to Cyclone Storage Phosphor screen (Packard Instrument Company, Inc., Meriden, CT). After 8 weeks of exposure for [^{3}H]DAMGO and 16 weeks of exposure for [^{3}H]DPDPE at 4°C, the images were detected by a Packard Cyclone Storage Phosphor System and analyzed by the analysis program Image Quant 3.3 (Molecular Dynamics).

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Autoradiography for D1 and D2 Dopamine Receptors

Dopamine D1 and D2 receptor densities were measured by quantitative ligand binding autoradiography according to Qian et al. (1992), with modifications. Briefly, the brain sections were preincubated at 4°C for 30 min in 50 mM Tris-HCI buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$, then incubated for 60 min in the same buffer with a final concentration at 0.4 nM [^{3}H]SCH23390 or 0.8 nM [^{3}H] spiperone in the presence of 100 nM ketanserin to prevent binding of the ligand to 5-HT receptors at room temperature. The brain sections were incubated with 30 μM (±)SKF38393 (Huang et al., 1997; Ongali et al., 2000; Tien et al., 2003) or 300 μM (±)sulpiride (Zeng et al, 2001; Zavitsanou and Huang, 2002) for nonspecific binding of D1 or D2 receptors, respectively. Labeled slides were placed in X-ray cassettes with a set of [^{3}H]-impregnated plastic standards ([^{3}H]Microscale RPA 510; Amersham Life Science) and juxtaposed to Kodak BioMax MS film. The [^{3}H1SCH23390 was exposed to film for 2 months (−80°C), and the [^{3}H]spiperone was exposed to film for 3 months (−80°C). The autoradiograms were analyzed via scanning densitometer (Personal Densitometer; Molecular Dynamics), operating under the image acquisition and analysis program Image Quant 3.3 (Molecular Dynamics).

Statistical Analysis

Data were expressed as mean ± SEM. μ-OR binding data were analyzed by one-way ANOVA, followed by a post hoc Student-Newman-Keuls multiple-comparisons test. A difference was considered significant at $P < 0.05$. PPE mRNA expression, δ-OR binding, and D1 and D2 dopamine receptor binding data were analyzed by two-way ANOVA (genotype vs. dose), followed by a post hoc Student-Newman-Keuls multiple-comparisons test. A difference was considered significant at $P < 0.05$.

RESULTS

PPE mRNA Expression

Two-way ANOVA revealed significant METH-induced effects on PPE mRNA expression in the striatum [genotype: $F(1,53) = 34.6$, $P < 0.001$; dose: $F(3,53) = 5.0$, $P < 0.005$; interactions: $F(3,53) = 11.7$, $P < 0.001$] and in the nucleus accumbens [genotype: $F(1,51) = 15.4$, $P < 0.001$; dose: $F(3,51) = 3.4$, $P < 0.05$; interactions: $F(3,51) = 5.0$, $P < 0.005$]. METH-treated wild-type mice showed a marked increase in PPE rnRNA expression in the striatum and nucleus accumbens at all administered doses (0.625, 2.5, and 10 mg/kg). The lowest dose of METH (0.625 mg/kg) produced a significant increase in PPE mRNA expression, which indicates that the alteration of PPE mRNA expression is very sensitive to METH treatment. METH at doses of 0.625 and 2.5 mg/kg resulted in a significant decrease in PPE mRNA expression in the striatum but not in the nucleus accumbens in μ-OR knockout mice. This decrease in PPE mRNA expression was reversed at the 10 mg/kg METH dose in μ-OR knockout mice. PPE mRNA expression in the striatum and nucleus accumbens of the METH (0.625 or 2.5 mg/kg)-treated μ-OR knockout mice was significandy lower than that of the corresponding wild-type controls (Table I).

μ- and δ-OR Ligand Binding

METH-treated wild-type mice showed a significant decrease in μ-OR ligand ([^{3}H]DAMGO) binding in the striatum [$F(3,24) = 5.3$, $P < 0.01$] at all administered METH doses, but not in the nucleus accumbens [$F(3,24) = 1.8$, $P = 0.17$; Table II]. This result may represent a compensatory consequence to METH-induced enhancement of PPE mRNA expression in the brains (Table I). No [^{3}H]DAMGO binding was detected in any of the brain regions

examined in μ-OR knockout mice (Table II). This result confirms that no functional μ-OR is expressed in the brain of μ–OR knockout mice (Loh et al., 1998).

Table III shows the quantitation of δ-OR ligand ([^{3}H]DPDPE) binding in the striatum [genotype: $F(1,53) = 26.7$, $P < 0.001$; dose: $F(3,53) = 41.1$, $P < 0.001$; interactions: $F(3,53) = 16.2$, $P < 0.001$] and nucleus accumbens [genotype: $F(1,50) = 60.7$, $P < 0.001$; dose: $F(3,50) = 19.1$, $P < 0.001$; interactions: $F(3,50) = 8.5$, $P < 0.001$] in the two genotypes of mice. In wild-type mice, METH caused a significant increase in ligand binding in die striatum at the 2.5 mg/kg dose. The data obtained revealed that [^{3}H]DPDPE binding was significantly increased and showed dose-dependent responses in both the striatum and the nucleus accumbens of μ-OR knockout mice following METH (0.625, 2.5, and 10 mg/kg) treatment (Table III).

D1 and D2 Dopamine Receptor Ligand Binding

Two-way ANOVA revealed significant effects of METH on D1 dopamine receptor ligand ([^{3}H]SCH23390) binding in the striatum [genotype: $F(1,52) = 5.9$, $P < 0.05$; dose: $F(3,52) = 7.2$, $P < 0.001$; interactions: $F(3,52) = 13.5$, $P < 0.001$] and in the nucleus accumbens [genotype: $F(1,49) = 5.9$, $P < 0.05$; dose: $F(3,49) = 1.6$, $P = 0.209$; interactions: $F(3,49) = 7.5$, $P < 0.001$]. In saline-treated control groups, μ-OR knockout mice showed significantly higher D1 dopamine receptor ligand binding in the striatum and nucleus accumbens compared with the wild-type mice. METH had no significant effect on D1 dopamine receptor ligand binding in the striatum and nucleus accumbens of wild-type mice. However, METH caused a significant decrease in ligand binding in the striatum at doses of 2.5 and 10 mg/kg and in the nucleus accumbens at all doses in μ-OR knockout mice (Table IV).

Two-way ANOVA revealed significant effects of METH on D2 dopamine receptor ligand ([^{3}H]spiperone) binding in the striatum [genotype: $F(1,45) = 2.4$, $P = 0.128$; dose: $F(3,45) = 22.6$, $P < 0.001$; interactions: $F(3,45) = 8.1$, $P < 0.001$] and in the nucleus accumbens [genotype: $F(1,51) = 1.0$, $P = 0.324$; dose: $F(3,51) = 23.3$, $P < 0.001$; interactions: $F(3,51) = 6.1$, $P = 0.001$]. In wild-type mice, METH caused a significant increase in ligand binding in the striatum at the 10 mg/kg dose and in the nucleus accumbens at doses of 0.625 and 10 mg/kg. In μ-OR knockout mice, METH treatment resulted in a decrease in ligand binding at a dose of 2.5 mg/kg and an increase in ligand binding at 10 mg/kg in both brain regions compared with the corresponding saline control groups. In saline and high-dose METH (10 mg/kg) groups, μ-OR knockout mice showed ligand binding higher than that of the wild-type mice in the striatum but not in the nucleus accumbens (Table V).

DISCUSSION

We have recently established a rodent model for the investigation of METH-induced behavioral sensitization (Chiu et al., 2005). In this model, mice receive daily i.p. injections of METH for 7 consecutive days to induce sensitization. Behavioral sensitization was then elicited with a single challenge dose of METH after four drug-abstinent days (day 11). Compared with the first injection, these mice showed significantly higher locomotor activity at low dose of METH and stereotyped behaviors at high dose of METH, which indicated that these animals have developed behavioral sensitization to METH. μ-OR knockout mice exhibited diminished behavioral sensitization to METH compared with wild-type controls (Shen et al., 2005). The neurochemical mechanisms responsible for this diminished behavioral sensitization to METH in μ-OR knockout mice remain unclear. In the present study, we investigated the changes in opioid and dopamine systems by using the same drug administration protocol as described above but without the challenge dose of METH on day 11.

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Repeated administration of psychostimulants has been implicated in augmented activity of the mesolimbic dopamine pathway in which marked alterations of the opioid system may have an important role (Herz, 1998). One of the most characteristic changes in the central opioid system caused by psychostimulants is an increase in gene expression of opioid peptides (Wang and McGinty, 1995, 1996). The present study shows that there is enhancement of PPE mRNA expression, a precursor of enkephalin, in the striatum and nucleus accumbens in METH-sensitized wild-type mice but not in μ-OR knockout mice. Previous studies have suggested that the dopamine receptor system is involved in the modulation of PPE mRNA expression. For example, chronic administration of D2 dopamine receptor agonist (Caboche et al., 1991; Pollack and Wooten, 1992) or D2 dopamine receptor antagonist (Tang et al., 1983) causes a decrease or an increase in PPE mRNA expression in the striatum, respectively. Denervation of presynaptic dopaminergic neurons by 6-hydroxydopamine (6-OHDA), -which induces postsynaptic supersensitivity, is associated with a significant increase in PPE mRNA expression in rats (Angulo et al., 1986; Gerfen et al., 1991). Moreover, this 6-OHDA-induced enhancement of PPE mRNA expression can be reversed by treatment with D2, but not D1 dopamine receptor agonists in rats (Gerfen et al., 1991), suggesting differential regulation of striatal PPE mRNA expression by D1 and D2 dopamine receptors. The regulation of striatal PPE mRNA is directly controlled by the D2- rather than by the D1-dopamine receptor system. However, the mechanism of regulation of METH (an indirect dopamine receptor agonist) on the expression of PPE mRNA remains unclear.

In contrast to PPE mRNA expression, there is a decrease in μ-OR ligand binding in the striatum in wild-type mice following METH exposure. As expected, no functional μ-OR was detected in μ-OR knockout mice in any of the METH treatment groups. The decreased μ-OR binding in wild-type mice may represent a compensatory response to METH-induced enhancement of PPE mRNA expression in the brain. Endogenous opioids not only modulate the central dopaminergic systems, they also appear to modulate the effects of drugs acting via these systems. Therefore, METH-induced changes in opioid peptide and μ-OR may contribute to the development of behavioral sensitization.

Lower δ-OR ligand binding, as determined with [^{3}H]DPDPE, was detected in the striatum and nucleus accumbens of μ-OR knockout mice compared with wild-type controls, suggesting a down-regulation of δ-OR binding in μ-OR knockout mice (Table III). These data are consistent with data reported by Kitchen et al. (1997). Behavioral studies have demonstrated a reduction in antinociceptive properties of δ-OR agonists in μ-OR knockout mice (Matthes et al., 1998). This partial reduction of δ-OR function may be related to the decrease of δ-OR binding in μ-OR knockout mice. METH treatment resulted in a significant increase in [^{3}H]DPDPE binding in the striatum and nucleus accumbens in μ-OR knockout mice but not in wild-type controls. The mechanism underlying this change remains to be investigated.

It has been reported that [A-Ala, D-Leu] enkephalin (DADLE), δ-opioid receptor peptide, can prevent METH-induced dopamine cell death; however, this protective effect of DADLE against METH can be reversed by naltrexone (Tsao et al., 1998). A possible explanation for the observed protective effect of DADLE on dopamine neurons might involve the activation of the δ-OR system, whereas the other OR systems are not activated. This hypothesis may explain our observation that METH induced a higher δ-OR ligand binding in the striatum and nucleus accumbens in mice lacking μ-OR. It is interesting to note that METH is associated with a dose-dependent increase in [^{3}H]DPDPE binding in these brain regions in μ-OR knockout mice but not in wild-type mice. The mechanism responsible for this change is unknown.

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Previous studies from our laboratory and other laboratories have demonstrated increases in mRNA expression (Park et al., 2001) and ligand binding (Tien et al., 2003; Lena et al., 2004) of D1 and D2 dopamine receptors in μ-OR knockout mice. The observed increase of D1 and D2 receptors in dopaminergic projection areas such as the striatum and nucleus accumbens could be a compensatory response to the absence of μ-OR on dopaminergic cell bodies in the substantia nigra and ventral tegmental area, where activation of the μ-OR is known to stimulate indirectly dopaminergic neurons projecting to these brain regions (Spanagel et al., 1992; Ozaki et al., 2002). In this study, saline-treated μ-OR knockout mice revealed patterns of D1 and D2 dopamine receptor binding similar to previous findings (Tien et al., 2003; Lena et al., 2004). METH treatment caused a significant decrease in brain D1 dopamine receptor binding in μ-OR knockout mice, but not in the wild-type mice, suggesting that the μ-OR plays a role in reducing METH-mediated influences on D1 dopamine receptors. The mechanism affording protection against METH to D1 dopamine receptors by μ-OR remains unclear.

It has been reported that supersensitivity of D2 dopamine receptors is related to locomotor hyperactivity and stereotyped behavior occurring after chronic treatment with METH (Ujike et al., 1990). Our previous study suggested that repeated administration of METH at 10 mg/kg induced significant stereotyped behaviors in both wild-type and μ-OR knockout mice (Shen et al., 2005). These findings suggest that METH-induced enhancement of D2 dopamine receptors in the striatum mediates the development of stereotyped behavior at higher doses of METH treatment. Additionally, the nucleus accumbens is a relatively small region in the mouse brain, making it difficult to identify the brain sections on which subsequent receptor binding analyses were performed. Therefore, there are significant variations in [^{3}H]spiperone binding in this brain region between samples, but no statistical difference was detected between wild-type mice treated with vehicle (control) and METH (2.5 mg/kg).

Long-term treatment with dopamine receptor antagonists (Hyttel, 1986), denervation of dopamine terminals with 6-OHDA (Graham et al., 1990), or prolonged depletion of dopamine stores with reserpine (Rubinstein et al., 1990) resulted in behavioral supersensitivity and an increased number of D2 dopamine receptors, without a change in the number of D1 dopamine receptors. Multiple doses of METH are reported to deplete dopamine levels in the brain (Schmidt et al., 1985; Gibb et al., 1990). The depletion of dopamine levels persists until the 95th day after METH administration (Itzhak et al., 2002). Consistently with published reports, our data show an increase in D2, but not in D1, dopamine receptor binding following depletion of dopamine by repeated exposure to METH. In μ-OR knockout mice, this balance between D1 and D2 dopamine receptors in the brains exposed to repeated METH has been altered. In other words, the μ-OR appears to play a role in modulation of the dopamine receptor system in METH-sensitized mice.

Taken together, METH increases PPE mRNA expression, decreases μ-OR levels, and increases D2 dopamine receptor levels but fails to change D1 dopamine receptor and δ-OR levels in the striatum and nucleus accumbens in wild-type mice. Most of these neurochemical changes found in wild-type mice are different in μ-OR knockout mice. The present study suggests that μ-OR is involved in the regulation of METH-induced changes in endogenous opioid peptide and dopamine receptors. However, the possibility of drug effects related not to behavioral sensitization but to repeated METH administration is not completely exclusive. Therefore, further study, such as acute METH administration, should be carried out.

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TABLE I

Quantitation of PPE mRNA Levels in METH-Sensitized Wild-Type and μ-OR Knockout Mice[†]

Brain region	Dose of METH (mg/kg)	Expression of PPE mRNAs (fmol/g tissue) Wild type	(μ-OR knockout
Striatum (Bregma: 1.62 mm)	0	262.59 ± 5.80	269.65 ± 4.14
	0.625	313.96 ± 17.26*	222.49 ± 10.99*,****
	2.5	343.93 ± 12.94*	186.41 ± 18.14*,****
	10	324.99 ± 14.91*	308.98 ± 24.66**,***
Nucleus accumbens (Bregma:1.62 mm)	0	231.55 ± 13.98	257.39 ± 19.74
	0.625	298.57 ± 12.45*	212.39 ± 10.27****
	2.5	299.45 ± 14.16*	197.27 ± 20.24****
	10	316.82 ± 23.08*	276.82 ± 27.74**,***

[†] Values are presented as mean ± SEM (eight mice per group). Statistical analysis was performed by two-way ANOVA, followed by Student-Newman-Keuls test.

* $P < 0.05$ compared with the respective saline-treated group.

** $P < 0.05$ compared with the 0.625 mg/kg-treated group.

*** $P < 0.05$ compared with the 2.5 mg/kg-treated group.

**** $P < 0.05$ compared with the corresponding wild-type mice.

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TABLE II

Quantitation of [^{3}H] DAMGO Binding to μ-OR in METH-Sensitized Wild-Type and μ-OR Knockout Mice[†]

Brain region	Dose of METH (mg/kg)	[^{3}H] DAMGO binding (fmol/mg tissue)	
		Wild type	μ-OR knockout
Shiatum (Bregma: 1.50 mm)	0	103.63 ± 8.46	Undetectable
	0.625	81.72 ± 5.73*	Undetectable
	2.5	75.57 ± 16.91*	Undetectable
	10	68.14 ± 5.11*	Undetectable
Nucleus accumbens (Bregma: 1.50 mm)	0	134.53 ± 8.34	Undetectable
	0.625	122.21 ± 15.8	Undetectable
	2.5	111.01 ± 5.32	Undetectable
	10	101.74 ± 10.0	Undetectable

[†] Values are presented as mean ± SEM (eight mice per group). Statistical analysis was performed by one-way ANOVA, followed by Student-Newman-Keuls test.

* $P < 0.05$ compared with the respective saline-treated group.

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TABLE III

Quantitation of [^{3}H]DPDPE Binding to δ-OR Binding in METH-Sensitized Wild-Type and μ-OR Knockout Mice[†]

		[^{3}H] DPDPE binding (fmol/mg tissue)	
Brain region	**Dose of METH (mg/kg)**	**Wild type**	**μ-OR knockout**
Striatum (Bregma: 1.58 mm)	0	69.69 ± 0.93	60.64 ±0.65[****]
	0.625	68.01 ± 1.64	63.37 ± 0.75[*]
	2.5	73.18 ± 0.53[*,**]	70.20 ± 0.30[*,**]
	10	71.36 ± 0.63[**]	74.95 ± 1.09[*,**,***]
Nucleus accumbens (Bregma: 1.58 mm)	0	73.55 ± 1.61	60.48 ± 0.91[****]
	0.625	71.77 ± 1.97	63.69 ± 0.76[*]
	2.5	76.62 ± 0.86[**]	70.23 ± 0.76[*,**]
	10	74.84 ± 0.89	74.63 ± 1.63[*,**,***]

[†]Values arc presented as mean ± SEM (eight mice per group). Statistical analysis was performed by two-way ANOVA, followed by Student-Newman-Keuls test.

[*]$P < 0.05$ compared with the respective saline-treated group.

[**]$P < 0.05$ compared with the 0.625 mg/kg-treated groups.

[***]$P < 0.05$ compared with the 2.5 mg/kg-treated group.

[****]$P < 0.05$ compared with the corresponding wild-type mice.

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TABLE IV

Quantitation of [^{3}H] SCH23390 Binding to D1 Dopamine Receptor in METH-sensitized Wild-Type and μ-OR Knockout Mice†

Brain region	Dose of METH (mg/kg)	[^{3}H] SCH23390 binding (fmol/mg tissue) Wild type	μ-OR knockout
Striatum (Bregma: 1.30 mm)	0	124.96 ± 7.15	150.97 ± 8.84***
	0.625	123.21 ± 4.82	137.77 ± 8.51
	2.5	145.16 ± 5.52	86.61 ± 6.19*,**
	10	122.66 ± 10.98	87.34 ± 5.81*,**
Nucleus accumbens (Bregma: 1.30 mm)	0	100.24 ± 7.24	132.61 ± 10.98***
	0.625	105.88 ± 7.96	97.56 ± 8.27*
	2.5	118.22 ± 6.87	79.34 ± 6.63*
	10	124.60 ± 16.82	73.58 ± 10.14*

†Values are presented as mean ± SEM (eight mice per group). Statistical analysis was performed by two-way ANOVA, followed by Student-Newman-Keuls test.

*$P < 0.05$ compared with the respective saline-treated group.

**$P < 0.05$ compared with the 0.625 mg/kg-treated group.

***$P < 0.05$ compared with the corresponding wild-type mice.

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TABLE V

Quantitation of [^{3}H] Spiperone Binding to D2 Dopamine Receptor in METH-Sensitized Wild-Type and μ-OR Knockout Mice[†]

		[^{3}H] spiperone binding (fmol/mg tissue)	
Brain region	**Dose of METH (mg/kg)**	**Wild type**	**μ-OR knockout**
Striatum (Bregma: 1.32 mm)	0	485.88 ± 13.39	519.19 ± 9.76****
	0.625	500.37 ± 6.87	476.65 ± 7.97*
	2.5	496.43 ± 5.98	470.59 ± 8.14*
	10	531.45 ± 14.77*	594.91 ± 10.28*,**,***,****
Nucleus accumbens (Bregma: 1.32 mm)	0	360.72 ± 16.59	384.52 ± 9.61
	0.625	433.17 ± 9.52*	384.52 ± 9.61****
	2.5	378.86 ± 10.03	338.61 ± 15.43*,****
	10	436.81 ± 13.33*	468.101 ± 7.73*,**,***

[†] Values are presented as mean ± SEM (eight mice per group). Statistical analysis was performed by two-way ANOVA, followed by Student-Newman-Keuls test.

* $P < 0.05$ compared with the respective saline-treated group.

** $P < 0.05$ compared with the 0.625 mg/kg-treated groups.

*** $P < 0.05$ compared with the 2.5 mg/kg-treated group.

**** $P < 0.05$ compared with the corresponding wild-type mice.