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Methylphenidate and μ opioid receptor interactions: A pharmacological target for prevention of stimulant abuse

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Abstract

Methylphenidate (MPH) is one of the most commonly used and highly effective treatments for attention deficit hyperactivity disorder (ADHD) in children and adults. As the therapeutic use of MPH has increased, so has its abuse and illicit street-use. Yet, the mechanisms associated with development of MPH-associated abuse and dependence are not well understood making it difficult to develop methods to help its mitigation. As a result, many ADHD patients especially children and youth, that could benefit from MPH treatment do not receive it and risk life-long disabilities associated with untreated ADHD. Therefore, understanding the mechanisms associated with development of MPH addiction and designing methods to prevent it assume high public health significance. Using a mouse model we show that supra-therapeutic doses of MPH produce

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Disclosure/Conflict of Interest

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1. High doses of methylphenidate upregulate activity of the μ opioid receptors and induce conditioned place preference.
2. Co-administration of naltrexone and methylphenidate prevents the μ opioid receptor upregulation and conditioned place preference.
3. The μ opioid receptor may be targeted pharmacologically to prevent stimulant abuse.

rewarding effects (surrogate measure for addiction in humans) in a conditioned place preference paradigm and upregulate μ opioid receptor (MOPR) activity in the striatum and nucleus accumbens, brain regions associated with reward circuitry. Co-administration of naltrexone, a non-selective opioid receptor antagonist, prevents MPH-induced MOPR activation and the rewarding effects. The MPH-induced MOPR activation and rewarding effect require activation of the dopamine D1 but not the D2 receptor. These findings identify the MOPR as a potential target for attenuating rewarding effects of MPH and suggest that a formulation that combines naltrexone with MPH could be a useful pharmaceutical approach to alleviate abuse potential of MPH and other stimulants.

Keywords

Methylphenidate; stimulants; dopamine receptor; opioid receptor; reward; reinforcement; addiction

Introduction

Methylphenidate (MPH) is one of the most commonly prescribed stimulants for treatment of attention deficit hyperactivity disorder (ADHD) in children and adults (Arnsten, 2006; Olfson et al., 2002; Zuvekas et al., 2006). Although therapeutic effects of MPH are well documented in over 100 studies of safety and efficacy in pediatric and adult ADHD (Brown et al., 2005), serious concerns persist about its negative side effects especially misuse, abuse and addiction. Studies in animal models show that high doses of MPH can produce reinforcement or reward [(behavioral measures in animals that are surrogate measures for addiction in humans) (Bergman et al., 1989; Botly et al., 2008; Gatley et al., 1996; Harvey et al.; Hiranita et al., 2009; Johanson and Schuster, 1975; Kuczenski and Segal, 2005; Marusich et al.; Meririnne et al., 2001; Wooters et al., 2011)]. Although not every preclinical study supports the view that MPH exposure can lead to reinforcement or reward, perhaps because the time of onset, dose and duration of MPH exposure differ among the studies (Andersen et al., 2002; Kuczenski and Segal, 2005; Robbins, 2002; Wilens et al., 2003), concerns about abuse potential of MPH persist in the clinical and lay communities alike. A manifestation of these concerns is the Schedule II status of MPH in the US Drug Enforcement Administration's drug scheduling scheme – in the same schedule as cocaine and morphine.

A number of studies suggest that rapid elevation of MPH levels in the blood and brain that occurs following intranasal or oral administration of supra-therapeutic doses is a key requirement for development of MPH associated euphoria, reinforcement and addiction (Ding et al., 2004; Gerasimov et al., 2000; Swanson and Volkow, 2003; Volkow et al., 2002). With that in mind, several slow- or extended-release formulations of MPH have been developed (e.g., Concerta®, Metadate®, Ritalin LA®). Clinical studies show that the subjective, positive effects of MPH (feelings of liking or euphoria) are significantly lower when the slow-release formulations are administered compared to the immediate release ones and that abuse potential of the former is likely to be low (Kollins et al., 1998; Parasrampur et al., 2007; Spencer et al., 2006). However, this pharmaceutical approach has not reduced MPH abuse because most abuse (whether MPH or other stimulants) occurs via intranasal administration of crushed preparations (Bright, 2008). Pulverization negates slow-release mechanisms and leads to rapid increases in brain MPH concentrations. Moreover, the immediate-release preparations continue to be in wide circulation, perhaps due to their lower cost. Thus, we face today the unfortunate reality that MPH abuse continues and may even be on the rise (DuPont et al., 2008; Setlik et al., 2009).

As a result of concerns about addiction potential of MPH among patients, families and even clinicians, many ADHD patients especially children and youth that could benefit from MPH treatment may not receive it. Untreated ADHD can have lifelong consequences including debilitating deficiencies in educational, occupational, and interpersonal functioning, with enormous cost to the individual, the family and our entire society (Matza et al., 2005; Matza et al., 2004a; Matza et al., 2004b). Therefore, attempts at developing abuse-free, therapeutically potent MPH assume high priority. Such attempts must begin with a full characterization of the CNS mechanism of action of MPH, distinction between mechanisms that mediate addiction *versus* therapeutic efficacy, selective targeting of the former by pharmacological means and finally, a strategy to circumvent pulverization of the preparation.

Although the principal molecular targets of MPH in the CNS are dopamine and noradrenaline, at sufficiently high doses MPH can also activate the μ opioid receptor (MOPR) in the brain (Crawford et al., 2007; Halladay et al., 2009; Wiley et al., 2009). Since reinforcing effects of highly addictive substances such as cocaine and heroin involve MOPR activation (Soderman and Unterwald, 2008; Zubieta et al., 1996), the reinforcing effects of high doses of MPH also may be mediated via MOPR activation. If this hypothesis is validated, it would follow that blocking the MOPR by using opioid receptor antagonists could alleviate the abuse potential of MPH. Previous reports have followed this line of research and found that in normal human volunteers combination of amphetamine (another stimulant that is effective as ADHD treatment and that also has significant abuse potential) and naltrexone, an opioid receptor antagonist, mitigates the subjective, positive effects (feelings of liking) of amphetamine (Jayaram-Lindstrom et al., 2008; Jayaram-Lindstrom et al., 2007; Jayaram-Lindstrom et al., 2004). Although these findings are highly promising, whether the mitigation of the subjective feelings translates into mitigation of addiction remains uncertain. Animal models are uniquely suited to test this possibility directly.

In the present study, we have used a mouse model to show that blocking the MOPR using naltrexone mitigates the rewarding effects of MPH. Thus, our findings link MPH with the brain opioid receptor system and highlight the potential for a novel pharmacological approach of combining naltrexone with MPH to attenuate abuse potential of MPH. Our findings reveal MOPR as a pharmacological target for developing an abuse-free formulation of MPH by combining it with naltrexone. Such a formulation could potentially overcome important drawbacks associated with slow-release MPH preparations because pulverization of the MPH + naltrexone formulation would not be an effective means of separating the two compounds.

Methods and Materials

Animals and materials

Adult C57BL/6 mice were purchased from Charles River Laboratories (Wilmington, MA). Only male mice were used. [35 S]GTP γ S (1250 Ci/mmol) was obtained from Perkin-Elmer Life and Analytical Sciences (Boston, MA). MPH, cocaine, naltrexone, DAMGO, SCH23390, Raclopride, GDP, GTP γ S, and PMSF were purchased from Sigma-Aldrich (St. Louis, MO).

Conditioned place preference (CPP)

A three-chamber place preference apparatus (Med Associates Inc., St. Albans VT, USA) was used. The apparatus has two equally sized (16.8X12cm) preference chambers connected by a central chamber (7.2X12cm), and is outfitted with sliding guillotine-style doors between each chamber. Photobeams connected to a computer system can record animal location and

time spent in that location. The central chamber has a gray colored smooth floor. The preference chamber is either white with a mesh floor or black with a bar floor. The CPP procedure included three phases: Preconditioning, conditioning and test phases.

The pre-conditioning phase was performed on day 1 (two sessions daily, AM and PM). In each preconditioning session, mice were initially placed in the central gray chamber for 2 min and then allowed free access to the white and black chambers for 20 min. The time spent in each chamber was recorded. For the next phase in the assay, the conditioning phase, the non-preferred chamber (i.e. the chamber in which less time was spent) was designated as the drug-paired chamber and the preferred chamber (i.e. the chamber in which more time was spent) was designated as the vehicle-paired chamber.

The conditioning phase was carried out on each of days 2 to 6. There were two conditioning sessions daily, morning session between 8 and 10 AM and afternoon session between 2 and 4 PM. There was one session each for vehicle-paired (saline as vehicle) and drug-paired (cocaine or MPH as drugs) conditions on each day of the conditioning phase. The mice were administered saline or drug (i.p.) in the saline- or drug-paired sessions, respectively and placed in the central gray chamber for 2 min (to isolate injection effect to the central chamber) and then confined to the vehicle- or drug-paired chamber, respectively for 30 min. On the first day of the conditioning phase, the mouse received saline during the morning session and the drug in the afternoon session. The next day, the order of treatments was reversed: drug in the morning session and saline in the afternoon session. We continued to alternate between these two paradigms for the remainder of the conditioning phase. This conditioning strategy was used for all the experiments except those using naltrexone. A different strategy was employed to account for the relatively long half-life of naltrexone [8-10 hr; (Akala et al., 2008; Cone et al., 1978; Misra et al., 1987; Verebey and Mule, 1975)] when naltrexone was used alone or when it was co-administered with MPH. In these experiments, we performed only one conditioning session per day (morning session between 8 and 10 AM). We administered saline on the first day and naltrexone (or naltrexone + MPH) the following day. We alternated between saline and naltrexone administrations for each of the next 9 days (total duration of conditioning was 10 days).

In the experiments using a drug co-administration strategy, naltrexone was administered 30 min prior to MPH and SCH23390 or raclopride were injected 10 min prior to MPH, in accordance with previous reports where these drugs were used (Meririnne et al., 2001).

During the test phase, (one session on the day after the conditioning phase) the mice were placed in the central gray chamber for 2 min and then given free access to the drug- and saline-paired chambers for 20 min. The time spent in each chamber was recorded. The difference between time spent in the drug-paired chamber during the test phase and pre-conditioning phases was calculated as the CPP score. Saline and the drugs were administered intraperitoneally. All the behavioral analyses were performed during the light phase of the light-dark cycle. We used between 6 and 12 mice in these experiments. The exact number of mice in each group for each experiment is specified in the Results section.

[³⁵S] GTPγS binding

Activity of the μ opioid receptor (MOPR) was assayed by using [³⁵S]GTPγS binding on membrane preparations using a modification of our previously described method (Zhu et al., 1997). The same mice that were used in the conditioned place preference assay were used for these assays also. Immediately upon completion of the behavioral assay, the mice were sacrificed by cervical dislocation and the brain was dissected rapidly. The brain was frozen in liquid nitrogen and 1.0 mm slices of the brain were prepared in the coronal plane using a tissue-slicing matrix (Model 15003; Ted Pella, Redding, CA). Caudate-putamen and nucleus

accumbens were identified in the frozen slices based on anatomical landmarks and samples from these two brain regions were collected using a tissue punch. The samples were homogenized using teflon pestle in Eppendorf tubes (10 strokes) in 300 μ l of homogenization buffer containing 25 mM Tris/pH7.4, 5 mM EDTA and 0.1 mM PMSF and kept on ice. Homogenate was diluted to 2.5 ml using the homogenization buffer and centrifuged at $\sim$ 350,000g for 30 min. After washing 3 times with 50 mM Tris-HCl/pH7.4, the pellets were re-suspended in 50 mM Tris-HCl/pH7.4 containing 0.32 M sucrose, passed through a 26.5G needle 3 times, frozen in dry ice/ethanol and stored in -80°C until use. DAMGO was used to stimulate MOPR. The membranes (10 μ g protein) were incubated in buffer (50 mM HEPES/pH 7.4, 100 mM NaCl, 5 mM MgCl_2 and 1 mM EDTA/pH8.0) containing [^{35}S]GTP γ S ($\sim$ 100,000 dpm, 80 pM) and 100 μ M GDP with or without DAMGO in a total volume of 0.5 ml for 60 min at 30°C . Nonspecific binding was defined by incubation in the presence of 10 μ M GTP γ S. Nonspecific binding was found to be similar in the presence or absence of agonist and was subtracted from total stimulated and total basal binding. Bound and free [^{35}S] GTP γ S were separated by filtration with GF/B filters under reduced pressure. Radioactivity on filters was determined by liquid scintillation counting. Nonspecific binding was subtracted from total stimulated and basal binding. The basal binding in the caudate putamen ranged between 45.9 ± 1.9 and 47.5 ± 3.5 fmol/mg protein, while that in the nucleus accumbens was between 88.7 ± 7.9 and 92.5 ± 9.3 fmol/mg protein. MOPR activity was reported as % of baseline (unstimulated) activity: [Disintegrations per minute (DPM) with agonist stimulated binding – DPM of nonspecific binding) / (DPM basal (without agonist) – DPM nonspecific) X 100. Protein content of membranes was determined by the BCA method of (Smith et al., 1985) with bovine serum albumin as the standard.

Data analysis

Differences between two experimental groups were analyzed for statistical significance by using Student's t-test. Treatment effects on multiple groups were tested for significance by using one-way ANOVA and the differences among the groups were tested for significance by using Dunnett's multiple comparisons test.

Results

High doses of MPH induce conditioned place preference

Reinforcing properties of MPH are influenced by the dose and route of administration, with parenteral high doses but not oral low doses leading to addiction (Gerasimov and Volkova, 1991; Kuczenski and Segal, 2002, 2005; Patrick and Markowitz, 1997). In a previous study, we established that 0.75mg/kg MPH administered to adult mice produced serum and brain concentrations of d-methylphenidate (the pharmacologically active isomer) that were equivalent to its serum and estimated brain concentrations in ADHD patients taking therapeutic doses of MPH (Balcioglu et al., 2009). Therefore, we considered 0.75mg/kg MPH as the low dose, potentially equivalent to the therapeutic dose in human subjects.

We used a conditioned place preference (CPP) paradigm to establish whether the high dose of MPH (7.5 mg/kg; 10 times the low dose) could produce rewarding effects in a mouse model. We used 3 drug stimuli: cocaine (10mg/kg) as a positive control drug because it reliably establishes reward in a CPP paradigm (Carelli, 2004; Wise, 1996), high dose MPH (7.5mg/kg), and low dose MPH (0.75mg/kg). Saline was used as a negative control. We found that the mice exposed to the high dose MPH showed significant place preference (Fig. 1A; t-test; $p=0.004$; $n=6$ /group), as did the mice exposed to cocaine (Fig. 1A; t-test; $p=0.005$, $n=6$) while the mice exposed to either the low dose MPH or saline did not (Fig. 1A; t-test, $p>0.05$). When the CPP scores were compared among the 4 groups using one-way-NOVA, the drug treatments were found to produce significant effects (Fig. 1B; $F=6.61$;

$p=0.003$; $n=6$ per group). Comparison among the groups showed that high dose MPH (7.5 mg/kg) and cocaine (10 mg/kg) groups had significantly higher CPP scores compared to the saline and low dose MPH (0.75 mg/kg) groups (Fig. 1B; multiple comparison test, $p<0.05$). There was no statistically significant difference in the CPP scores between the high dose MPH and cocaine groups or the low dose MPH and saline groups (Fig. 1B; multiple comparison test, $p>0.05$). Thus, under these experimental conditions, high dose MPH was essentially as rewarding as cocaine whereas the low dose and saline were equally ineffective.

High doses of MPH enhance μ opioid receptor activity

Opioid receptors in the brain fall into 3 types: Mu (μ), delta (δ) and kappa (κ). The caudate-putamen, nucleus accumbens, frontal cortex and ventral midbrain, all of which are intricately involved in the reward and addiction circuitry, are enriched in these receptors (Trigo et al., 2010). Each receptor is believed to facilitate different aspects of reward circuits via interactions with opioids and neurotransmitters including dopamine (Trigo et al., 2010). Activation of the μ opioid receptor (MOPR) is associated with euphoria and reward whereas activation of the κ opioid receptor (KOPR) is associated with dysphoria and aversion (Trigo et al., 2010). Since upregulation of the MOPR is generally associated with rewarding effects - e.g. following cocaine exposure (Soderman and Unterwald, 2008; Zubieta et al., 1996) we determined if the 7.5 mg/kg MPH dose that produced CPP also upregulated MOPR activity. We assayed [35 S]GTP γ S binding induced by the selective MOPR agonist DAMGO using membrane preparations from the caudate-putamen and nucleus accumbens of mice that had received MPH (0.75 mg/kg or 7.5 mg/kg), cocaine (10 mg/kg) or saline intraperitoneally in the CPP assay. Initial experiments showed that maximal [35 S]GTP γ S binding was achieved at 10 μ M DAMGO (Fig. 2A). Therefore, this concentration of DAMGO was used in all the subsequent experiments (Fig. 2B). One-way ANOVA revealed significant effect of the drug treatment on DAMGO stimulated [35 S]GTP γ S binding in the caudate putamen ($F=5.89$; $p=0.005$; $n=6$ per group) and the nucleus accumbens ($F=4.345$, $p=0.016$; $n=6$ per group). The basal [35 S]GTP γ S binding (i.e. unstimulated binding) was not significantly different among the different groups. Comparison among the experimental groups showed that the increase in DAMGO-stimulated [35 S]GTP γ S binding (compared to the basal levels) in the caudate-putamen and nucleus accumbens of mice exposed to 7.5 mg/kg MPH or cocaine was significantly greater than that in the mice exposed to saline (Fig. 2B; $p<0.05$). Low dose (0.75 mg/kg) MPH did not produce significant enhancements ($p>0.05$) in DAMGO-stimulated [35 S]GTP γ S binding.

Prior exposure to opioid receptor antagonist attenuates high dose MPH induced CPP

Since the high dose MPH-induced CPP is associated with MOPR activation, we examined if an opioid receptor antagonist could attenuate MPH-induced CPP. We performed CPP assays in which naltrexone, a non-selective opioid antagonist, was administered 30 min prior to MPH (7.5 mg/kg) at 1, 5 or 10 mg/kg. As controls, we used saline, MPH (7.5 mg/kg) alone or 1 and 10 mg/kg naltrexone alone. As predicted, MPH (7.5 mg/kg) on its own induced CPP (Fig. 3A; t-test, $p=0.00002$, $n=9$ per group). When naltrexone 1, 5 or 10 mg/kg was administered 30 min prior to MPH, rewarding effects did occur (Fig. 3A; t-tests; 1 mg naltrexone+MPH, $p=0.003$, $n=11$; 5mg naltrexone+MPH, $p=0.009$, $n=7$; 10 mg naltrexone+MPH, $p=0.001$, $n=7$). However, the CPP scores under these conditions were significantly lower than those when MPH was administered alone (see below). Naltrexone alone did not induce CPP at 1 or 10 mg/kg (Fig. 3A, t-tests, in each case, $p>0.05$ and $n=6$). We did not evaluate CPP following administration of 5 mg/kg naltrexone alone. One-way ANOVA of the data on CPP scores (Fig. 3B) showed significant effects of the drug treatment ($F=9.71$; $p<0.001$). Multiple comparison test confirmed that the CPP score produced by the high dose MPH (7.5 mg/kg) was significantly greater than that produced by the saline control

($p < 0.01$). However, the CPP scores of neither the 5 mg naltrexone + MPH nor the 10 mg naltrexone + MPH groups were significantly different (Fig. 3B; $p > 0.05$) from the saline control groups suggesting that these two doses of naltrexone inhibited high dose MPH-induced place preference. The CPP scores for the 1 mg naltrexone + MPH group were significantly higher than the saline control group (Fig. 3B; $p < 0.01$). These data show that blocking opioid receptors using naltrexone prior to MPH administration can significantly attenuate rewarding effects of MPH.

Prior exposure to opioid receptor antagonist attenuates high dose MPH induced upregulation of MOPR activity

To verify that the naltrexone-induced reduction in the CPP score was indeed due to blockade of the MOPR by naltrexone, we analyzed MOPR activity following the CPP assay in which MPH+naltrexone combinations were employed. At the end of the CPP assay, we collected the caudate-putamen and nucleus accumbens from each group of mice and assayed MOPR activity by using [35 S]GTP γ S binding. The basal [35 S]GTP γ S binding was not significantly different among the different groups. However, one-way ANOVA revealed significant effects of the drug treatment in the caudate putamen (Fig. 3C; $F = 66.17$, $p < 0.001$; $n = 8$) and the nucleus accumbens (Fig. 3C; $F = 45.88$, $p < 0.001$; $n = 8$). Multiple comparison analysis showed that the MOPR activity was significantly reduced in the caudate-putamen and nucleus accumbens of mice that had received 10 mg/kg naltrexone prior to 7.5 mg/kg MPH compared to the mice that had received 7.5 mg/kg MPH alone (Fig. 3C; $p < 0.01$). Comparison between the saline controls and high dose MPH (7.5 mg/kg) groups showed significant upregulation of the MOPR activity in the MPH group in both the brain regions (Fig. 3C).

Dopamine D1-receptor antagonist attenuates high dose MPH induced CPP

Earlier reports suggested that in rats, MPH-induced CPP requires dopamine D1-receptor activation (Meririnne et al., 2001). These earlier data together with our present findings suggest a mechanistic link between MPH, dopamine, MOPR and CPP. To characterize this link further, we examined whether the CPP induced by high dose MPH could be blocked by prior exposure to dopamine D1- or D2-receptor antagonists. We used the D1-receptor antagonist SCH23390 (0.2 mg/kg) and the D2-receptor antagonist raclopride (0.5 mg/kg). These doses of the receptor antagonists had been used previously in a rat model of MPH-induced CPP (Meririnne et al., 2001). As described earlier, high doses of MPH (7.5 mg/kg) produced CPP (Fig. 4A; t-test; $p = 0.0001$, $n = 9$) whereas administration of SCH23390 prior to MPH prevented development of the CPP (Fig. 4A, t-test, $p > 0.05$ $n = 6$). Administration of raclopride 10 min prior to MPH did not affect MPH-induced CPP (Fig. 4, t-test; $p = 0.0005$, $n = 7$). SCH23390 or raclopride alone did not produce CPP (Fig. 3A; t-test, $p > 0.05$, $n = 6$ for each drug treatment). When we compared CPP scores among the different groups by one-way ANOVA significant effects of drug treatment were found (Fig. 4B, $F = 9.3$, $p < 0.0001$). Multiple comparison tests revealed that the CPP scores were significantly higher in the MPH and raclopride+MPH groups compared to the saline group ($p < 0.01$) while comparisons among the saline and SCH23390 alone, raclopride alone or SCH23390+MPH groups did not show significant differences (Fig. 3B; $p > 0.05$).

Dopamine D1-receptor antagonist attenuates high dose MPH induced upregulation of MOPR activity

Since MOPR activation was required for MPH-induced CPP, we suggest that high dose MPH-induced activation of the D1-receptor led to activation of MOPR and development of the CPP. To test this possibility and determine if the D1-receptor antagonist-induced reduction in the CPP score correlated with blockade of the MOPR, we analyzed MOPR activity following the CPP assay in which MPH+SCH23390 or MPH+raclopride

combinations were employed. At the end of the CPP assay, we sacrificed all the mice, collected the caudate-putamen and nucleus accumbens from each group of mice, and assayed MOPR activity by using [^{35}S]GTP γ S binding. One-way ANOVA revealed significant effects of the drug treatment on MOPR activity in the caudate putamen (Fig. 5A; $F=6.67$; $p<0.001$) and the nucleus accumbens (Fig. 5B; $F=4.34$; $p=0.003$). Multiple comparison test revealed that the MOPR activity was significantly upregulated in the mice receiving MPH alone and raclopride + MPH compared to that in the mice receiving saline both in the caudate putamen (Fig. 5A; $p<0.05$; saline $n=8$, MPH $n=8$, raclopride+MPH $n=7$) and the nucleus accumbens (Fig. 5B; $p<0.05$). However, the MOPR activity in the mice that had received SCH23390+MPH was not significantly different ($P>0.05$) compared to the mice that had received saline in the caudate putamen (Fig. 5A) or nucleus accumbens (Fig. 5B). Similarly, SCH23390 alone or raclopride alone did not affect MOPR activity compared to the saline treatment in either brain region. These data suggest that the D1-receptor antagonist not only reduced the CPP score but also blocked high dose MPH-induced activation of the MOPR. The D2 receptor antagonist did not affect either of these two phenomena.

Discussion

Our data show that high doses of MPH administered intraperitoneally enhance MOPR activity in the caudate putamen and nucleus accumbens and induce CPP. Administration of the non-selective opioid receptor antagonist naltrexone prior to MPH administration prevents the MPH-induced CPP and upregulation of MOPR. Collectively, these observations suggest the MOPR as a pharmacological target for designing abuse-free MPH formulations by combining naltrexone with MPH. Since naltrexone is already FDA-approved for the treatment of alcohol dependence (Cowen and Lawrence, 2006), the translational potential of our findings could be very high.

Although interactions between MPH and the brain opioid system have been reported previously (Crawford et al., 2007; Halladay et al., 2009; Wiley et al., 2009), our findings break new ground because they reveal MOPR as a pharmacological target for mitigating abuse potential of MPH. Previous studies in normal human volunteers tested the benefits of amphetamine + naltrexone combination on minimizing the subjective, positive effects (feelings of liking or euphoria) of amphetamine (Jayaram-Lindstrom et al., 2008; Jayaram-Lindstrom et al., 2007; Jayaram-Lindstrom et al., 2004). Whether these subjective feelings of euphoria may be forerunners of addiction is difficult to establish in human subjects. Animal models, such as those used here are uniquely suited to test this possibility. Therefore, our studies using a mouse model go beyond the human studies in unequivocally establishing that rewarding effects of high doses of MPH are associated with MOPR activation and that blocking the MOPR can mitigate the reinforcing effects of MPH.

Our findings also offer additional insights into the mechanisms mediating MPH-MOPR interactions. MPH is not known to directly activate MOPR. Previous reports suggested that dopamine D1 and D2 receptors are involved in the development of sensitization to MPH (Meririnne et al., 2001). Since sensitization is believed to play a key role in the development of reinforcement, reward and addiction (Robinson and Berridge, 1993, 2008), we hypothesized that high dose MPH-induced rewarding effects in our mouse model may involve MOPR activation via activation of the dopamine receptors. We found that dopamine D1 receptor activation may be an essential step in the activation of MOPR by MPH. Thus, dopamine receptor signaling may play critical roles in both the therapeutic and negative effects of MPH. The mechanisms underlying the differences between the actions of D1 and D2 receptors in MPH sensitization remain unclear (Meririnne et al., 2001). It is possible that the two receptors differentially affect acquisition *versus* expression of the place preference.

For example, D1-receptor antagonists but not D2-receptor antagonists are known to block acquisition of cocaine- and MPH-induced place preference (Baker et al., 1998; Baker et al., 1996; Cervo and Samanin, 1995; Meririnne et al., 2001; Mithani et al., 1986; Nazarian et al., 2004). Moreover, blocking both D1- and D2-receptors but not either D1- or D2-receptor alone prevents expression of cocaine place preference (Cervo and Samanin, 1995; Liao et al., 1998) suggesting that different mechanisms may mediate acquisition *versus* expression of place preference.

Another issue raised by our findings is the potential interaction between MOPR and dopamine receptors in the mediation of MPH-induced place preference. MOPR co-localizes with the D1 receptor, substance P and dynorphin in the striatal GABAergic medium spiny neurons (Georges et al., 1999; Guttenberg et al., 1996; McGinty, 2007), which contribute to the striatonigral (direct) pathway. MOPR co-localizes with the D2 receptor mainly in the striatal enkephalinergic GABA neurons (Ambrose et al., 2004; McGinty, 2007), which play an important role in the striatopallidal (indirect) pathway. Whether such differential localization of MOPR, D1- and D2-receptor contributes to the different roles of the two types of dopamine receptors in mediating MPH place preference remains to be determined.

Despite the promise offered by our findings for development of a potential abuse-free MPH formulation, a critical question that remains unanswered is whether the naltrexone + MPH combination can preserve the therapeutic effects of MPH on attention. Equally significantly, the dose of naltrexone that was found to be effective in preventing high dose MPH induced CPP and MOPR activation in the present study is higher than the dose typically used in the clinic for the treatment of addiction and dependence (1-2 mg/kg). At high doses naltrexone may block other opioid receptors and may also have negative side effects such as liver toxicity and dysphoria. However, selective MOPR antagonists do not cross the blood-brain barrier (CTOP, CTAP), limiting their translational uses. Thus, naltrexone remains the opioid receptor antagonist of choice for the purposes of developing an abuse-free MPH formulation.

We point out that higher doses of naltrexone may have been necessary in the present study because the dose of MPH used here was also much higher than the minimal dose needed to produce CPP (Kuczenski and Segal, 2005; Meririnne et al., 2001). Clearly, additional studies will be needed to establish a lower dose of naltrexone that can block rewarding effects of MPH and MPH-induced MOPR upregulation, preserve therapeutic potency of MPH, and will be free from potential side effects.

We point out that recent developments in drug delivery technology make it possible to combine higher doses of naltrexone with MPH to prevent MPH abuse without the negative side effects of naltrexone in patients taking MPH orally for therapeutic purposes (Aversion® Technology, Acura Pharmaceuticals, Palatine, IL.). A capsule can be designed in which an insoluble core of naltrexone is encased within a soluble shell of MPH so that when the capsule is taken orally, as prescribed, only the MPH is absorbed and the naltrexone core passes through the gastro-intestinal tract intact. However, if the capsule is crushed (for abuse), the naltrexone is released and would mitigate the abuse potential of MPH. The advantage of this preparation is that when the capsule is used therapeutically, the naltrexone is not absorbed into the system alleviating concerns about potential side effects of high doses of naltrexone, including actions at kappa and delta opioid receptors, as well as naltrexone's potential interaction with therapeutic actions of MPH.

Finally, we administered naltrexone 30 min prior to the administration of MPH. The two drugs were not administered together. The abuse-free formulation of MPH envisaged by us would have MPH and naltrexone combined together. However, neither the present study nor

the previous studies with amphetamine (Jayaram-Lindstrom et al., 2008; Jayaram-Lindstrom et al., 2007; Jayaram-Lindstrom et al., 2004) indicate unequivocally if co-administration of naltrexone and MPH can be as effective in mitigating reinforcing effects of MPH as successive administration of the two drugs.

We suggest that our findings with MPH likely apply to the entire class of stimulant drugs, including amphetamine, as well as analeptics such as modafinil because all of these compounds share a common dopaminergic mode of action and likely affect the MOPR in the same manner as MPH (Cenci et al., 1998; Sivam, 1989; Xia et al., 2007). An advantage of the naltrexone + stimulant combination envisaged here over the slow-release formulations, which happen to represent the only strategy on the market currently, is that the naltrexone + stimulant combination would not become ineffective upon pulverization, because pulverization alone cannot separate the naltrexone from the stimulant compound. Therefore, such formulation would be resistant to abuse by the most common form, namely intranasal administration of the pulverized preparation (Bright, 2008).

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Figure1.

High doses of methylphenidate (MPH) induce conditioned place preference (CPP; A, B). Mice exposed to cocaine or high-dose MPH (7.5 mg/kg) spent significantly longer period of time in the drug-paired chamber during the Test sessions compared to the pre-conditioning (PC) sessions whereas the mice exposed to saline or low dose MPH (0.75 mg/kg) did not show significant differences in this measure. Multiple comparisons test showed significant ($p < 0.05$) effects of cocaine and high dose MPH treatment on the CPP score compared to the score in the saline-treated group. There was no significant difference between the saline and low dose MPH groups or between the cocaine and high dose MPH groups.

Figure 2.

High doses of MPH upregulate μ opioid receptor (MOPR) activity in the caudateputamen and nucleus accumbens. $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding in membrane preparations from the caudate-putamen was increased by the selective MOPR agonist DAMGO in a concentration-dependent manner with an EC_{50} of ~ 1 and 0.1 mM (A). The maximal binding, which represented 1.75-fold of the basal level was reached at 10 mM concentration (arrow), which was the concentration used in the bindings assays (B). Multiple comparisons analysis showed that the cocaine and high dose MPH (7.5 mg/kg) groups showed significant increases in MOPR activity compared to the saline group ($p < 0.05$) in both the brain regions. There was no significant difference between the saline and low dose MPH (0.75 mg/kg) groups or between the cocaine and high dose MPH groups in either brain region.

Figure 3.

Naltrexone (5 or 10 mg/kg) administration prior to high dose (7.5 mg/kg) methylphenidate (MPH) reduces MPH-induced conditioned place preference (CPP; A and B) and MPH-induced activation of the μ opioid receptor (MOPR; C). Mice exposed to high-dose MPH plus saline spent significantly longer period of time in the drug-paired chamber during the Test sessions compared to the pre-conditioning (PC) sessions (A). Neither saline alone nor naltrexone (1 or 10 mg/kg) alone produced significant changes in this measurement. When naltrexone (1, 5 or 10 mg/kg) was administered prior to MPH, in each case there was a significant difference between PC and Test sessions indicating that each drug treatment had induced CPP. Multiple comparisons analysis of the CPP scores showed that prior treatment with 1, 5 or 10 mg/kg naltrexone significantly decreased the CPP score compared to prior treatment with saline (B). The decrease in the CPP score was naltrexone dose-dependent. In fact, the CPP score for the group that had received 5 mg/kg or 10 mg/kg naltrexone prior to MPH was not significantly different from that for the saline group (B). When we analyzed MOPR activity in the same 4 groups of mice, by using multiple comparisons analysis, we found that the MOPR activity in the MPH-treated group was significantly higher than that in the saline group. MOPR activity in the MPH+naltrexone (10 mg/kg) group was significantly lower than that in the saline or MPH groups.

Figure 4.

The dopamine D1-receptor antagonist SCH23390 but not the D2-receptor antagonist raclopride blocked high dose (7.5 mg/kg) methylphenidate (MPH) induced conditioned place preference (CPP). We performed CPP assay using saline, SCH23390, raclopride, or using a combination of MPH+saline, MPH+SCH23390 and MPH+raclopride. MPH plus saline and MPH+ raclopride and no other drug treatment produced significant increase in the time spent in the drug-paired chamber during the Test sessions compared to the pre-conditioning (PC) sessions (A). Multiple comparisons test showed that the MPH +SCH23390 group had significantly lower CPP score ($p<0.01$) compared to the MPH-only group and that there was no statistically significant difference between saline and MPH +SCH23390 groups. The MPH+raclopride group did not show significant differences from the MPH alone group.

Figure 5.

The dopamine D1-receptor antagonist SCH23390 but not the D2-receptor antagonist raclopride blocked the methylphenidate (MPH; 7.5 mg/kg) induced increase in the activity of the μ opioid receptor (MOPR) in membrane preparations from the caudate-putamen (A) and nucleus accumbens (B). Multiple comparisons analysis showed that MOPR activity was significantly higher in the MPH group compared to the saline group in the caudate putamen and nucleus accumbens. SCH23390+MPH administration reduced the MOPR activity to the level seen following the saline administration in both the brain regions. However, there was no significant difference between the MOPR activity induced by raclopride+MPH and MPH alone in either region. The MOPR activity induced when SCH23390 or raclopride were administered alone was not significantly different from that induced by the saline administration.