Behavioral and Molecular Effects of Dopamine D\textsubscript{1} Receptor Stimulation during Naloxone-Precipitated Morphine Withdrawal

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Morphine dependence is characterized by somatic and motivational signs of withdrawal that likely contribute to the maintenance of addictive behavior. The nucleus accumbens (NAc) receives extensive dopaminergic input and is an important substrate for mediating these aversive states. In the NAc, the function of the transcription factor cAMP response element binding protein (CREB) and AMPA glutamate receptor subunit, type 1 (GluR1) can be regulated by dopamine (DA) D\textsubscript{1} receptor-mediated phosphorylation (P-CREB, P-GluR1). However, the roles of D\textsubscript{1} receptors, CREB, and GluR1 in morphine dependence are not well understood. Here, we show that somatic signs of naloxone-precipitated withdrawal were associated with increased P-CREB, but not P-GluR1, in the NAc of morphine-dependent rats. The D\textsubscript{1} receptor agonist chloro-APB hydrobromide (SKF 82958) was rewarding in morphine-dependent rats and blocked naloxone-induced place aversions and somatic signs of withdrawal. Surprisingly, SKF 82958 increased P-GluR1, but not P-CREB, in the NAc, and naloxone reduced SKF 82958-mediated P-GluR1 induction specifically in morphine-dependent rats. Together, these results confirm that aversive treatments can increase CREB function in the NAc. Furthermore, they suggest a dependence-associated shift in the molecular mechanisms that regulate the consequences of D\textsubscript{1} receptor stimulation, favoring activation of GluR1 rather than CREB. These data raise the possibility that the rewarding effects of SKF 82958 in morphine-dependent rats involve increased P-GluR1 in the NAc, although the involvement of other brain regions cannot be ruled out. Regardless, these findings suggest for the first time that D\textsubscript{1} agonists might be useful for the treatment of withdrawal symptoms that contribute to the maintenance of opiate addiction in humans.

Key words: nucleus accumbens; CREB; GluR1; AMPA; place conditioning; SKF 82958

Introduction

Opiate withdrawal causes somatic abstinence signs as well as a motivational syndrome characterized by dysphoria and depression (Koob and Le Moal, 1997; De Vries and Shippenberg, 2002) in humans. These negative affective states are thought to contribute to addictive behaviors (Koob and Le Moal, 1997; Markou et al., 1998). The nucleus accumbens (NAc), which receives extensive dopaminergic input, plays a key role in opiate reward (Wise, 1989) as well as the motivational and somatic signs of opiate withdrawal (Koob et al., 1992; Harris and Aston-Jones, 1994). The opioid receptor antagonist naloxone precipitates physical withdrawal signs in morphine-dependent rats and produces behaviors, such as conditioned place aversions, that reflect aversive states mediated within the NAc (Stinus et al., 1990).

Acute morphine decreases formation of cAMP (Childers, 1991), whereas chronic morphine causes super-sensitivity of cAMP pathways in areas including the NAc (Nestler and Aghajanian, 1997). Precipitated opiate withdrawal unmasks upregulated cAMP pathways, which likely contribute to aversive states. Hypothetically, activation of dopamine (DA) D\textsubscript{1} receptors, which are positively coupled to adenylyl cyclase (Dearry et al., 1990), during morphine withdrawal could potentiate cAMP-mediated signaling (Chartoff et al., 2003a,b) and exacerbate aversive states. However, the role of DA and related signaling pathways in the NAc in morphine dependence is not well understood. Morphine withdrawal decreases DA in the NAc (Pothos et al., 1991; Diana et al., 1999), and administration of the nonspecific DA agonist apomorphine blocks signs of naloxone-precipitated withdrawal (Harris and Aston-Jones, 1994; Laviolette et al., 2002). Paradoxically, nonspecific dopamine agonists also block naloxone-induced place aversions (Bechara et al., 1998). In contrast, specific D\textsubscript{2} receptor agonists elicit withdrawal signs in morphine-dependent rats (Harris and Aston-Jones, 1994; Funada and Shippenberg, 1996), whereas a D\textsubscript{1} antagonist establishes place aversions in morphine-dependent and nondependent rats (Funada and Shippenberg, 1996).

Morphine-induced upregulation of cAMP-mediated signaling pathways likely causes intracellular adaptations including activation of cAMP-dependent protein kinase A (PKA) and phosphorylation of downstream targets including cAMP response element binding protein (CREB) and AMPA glutamate receptor subunit, type 1 (GluR1). Elevated CREB or GluR1 function in the
NAc appears associated with negative motivational states. For example, viral vector-mediated elevations of CREB or GluR1 within the NAc produce depressive- and aversive-like states in rodents, and reduced drug reward (Carlezon et al., 1998; Kelz et al., 1999; Pliakas et al., 2001). Elevation of GluR1 expression in the NAc reduces drug-seeking behavior (Sutton et al., 2003), an effect also caused by acute drug withdrawal (Stewart and Wise, 1992; Carlezon and Wise, 2003). In contrast, targeted disruption of CREB α and Δ isoforms (Maldonado et al., 1996; Walters and Blendy, 2001) or knock-out of GluR1 (Vekovischeva et al., 2001) in mice reduces the signs of opiate withdrawal. However, there is also evidence that increased glutamate signaling at AMPA receptors in the NAc contributes to the expression of psychomotor sensitization and drug seeking (Piech et al., 1996; Cornish and Kalivas, 2000), behaviors classically associated with increased motivational impact of drugs of abuse (Robinson and Berridge, 2001). Thus, the relationship between drug-related motivational states and CREB or GluR1 expression in the NAc are not well understood, particularly in animals with drug-induced alterations in intracellular signaling.

D1 receptor-mediated phosphorylation of CREB and GluR1 enhances their function (Chao et al., 2002b; Song and Huganir, 2002; Carlezon et al., 2005). Considering that morphine withdrawal increases the function of downstream targets of cAMP signaling in the striatum and NAc (Nye and Nestler, 1996; Gracy et al., 2001; Shaw-Lutchman et al., 2002) and triggers aversive withdrawal signs, we examined whether precipitated morphine withdrawal would activate CREB and GluR1 within the NAc. We also examined whether D1 agonist-induced stimulation of cAMP signaling during precipitated morphine withdrawal would further increase CREB and GluR1, thereby exacerbating withdrawal signs. Unexpectedly, we discovered dependence-mediated shifts in the consequences of cAMP-mediated signaling.

Materials and Methods

Rats. A total of 261 male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) were used in this study. Rats weighed 325–375 g at the time of the experiments and were maintained on a 12 h light/dark (7:00 A.M. to 7:00 P.M.) cycle with ad libitum access to food and water except during testing. Experiments were conducted in accordance with the 1996 National Institutes of Health Guide for the Care and Use of Laboratory Animals and McLean Hospital policies.

Drugs. Naloxone HCl and chloro-APB hydrobromide (SKF 82958) were obtained from Sigma-Aldrich (St. Louis, MO). Lithium chloride (LiCl) was obtained from Fisher Scientific (Pittsburgh, PA). Naloxone was dissolved in 0.9% saline (NaCl) and LiCl, and SKF 82958 was dissolved to rats in a volume of 1 ml/kg. Placebo and morphine pellets (75 mg morphine base/pellet) were obtained from the National Institute on Drug Abuse (Bethesda, MD). Rats were made dependent on morphine and killed 5 days after implantation 30 mg/kg morphine base/pellet, with or without LiCl were not implanted with pellets. Brains were rapidly removed and frozen in isopentane kept on dry ice. Brains were then sliced on a cryostat (HM 505 E; Microm, Walldorf, Germany) and kept at −20°C until the rostral NAc and caudate putamen (CPU) were used as 2.20 mm anterior to bregma. Bilateral tissue punches (1 mm2) were obtained from the NAc and CPU and placed in Eppendorf (Hamburg, Germany) tubes kept on dry ice. Tissue was sonicated (Sonics Dismembrator 60; Fisher Scientific) in 100 μl of 1% SDS to break apart cell membranes. Protein content was determined using the Bio-Rad (Hercules, CA) DC Protein Assay kit, and the concentration of each sample was adjusted to 2.0 mg/ml protein. NuPAGE lithium dodecyl sulfate sample buffer (Invitrogen, Carlsbad, CA) and 50 mM dithiothreitol were added to each sample before heating at 70°C for 10 min. Twenty micrograms of each sample were then loaded onto NuPAGE Novex 4–12% Bis-Tris gels (Invitrogen) for separation by gel electrophoresis. Proteins were subsequently transferred to polyvinylidene fluoride membrane (PerkinElmer Life Sciences, Boston, MA). Non-specific binding sites on the membranes were blocked for 2 h at room temperature in blocking buffer [5% nonfat dry milk in PBS and 0.1% Tween 20 (PBS-T)]. Blots were then incubated in primary antibody [1:4000 monoclonal anti-Ser383 phospho-CREB (P-CREB), 1:4000 anti-CREB (Cell Signaling Technology, Beverly, MA), 1:3000 anti-Ser445 phospho-GluR1 (P-GluR1), or 1:2000 anti-GluR1 (Chemicon, Temecula, CA)] in PBS-T overnight at 4°C. Blots were washed four times.
Results

Motivational and somatic morphine withdrawal signs can be dissociated by naloxone dose

Naloxone-induced place aversions in the place conditioning paradigm depended on an interaction between conditioning (pre-conditioning vs postconditioning) and dependence (placebo vs morphine) \( F_{(1,54)} = 17.136; p < 0.001 \), with repeated measures on conditioning (Fig. 1A). Simple main effects tests reveal that morphine-dependent rats displayed significant place aversions in response to all doses of naloxone tested \((0.01 \text{ mg/kg}; F_{(1,9)} = 9.272; p < 0.05; 0.1 \text{ mg/kg}; F_{(1,10)} = 74.841; p < 0.001; 1.0 \text{ mg/kg}; F_{(1,7)} = 24.198, p < 0.01 \), whereas placebo-implanted control rats only showed place aversions in response to the highest dose of naloxone \( F_{(1,6)} = 20.239; p < 0.01 \). The effect of naloxone on somatic withdrawal behaviors depended on an interaction between dose and dependence \( F_{(3,42)} = 3.447; p < 0.05 \), with repeated measures on dose (Fig. 1B). All doses of naloxone caused significant increases in somatic withdrawal behaviors in morphine-dependent rats compared with morphine-dependent rats treated with vehicle \( F_{(3,21)} = 8.837; p < 0.001 \), whereas naloxone did not significantly increase somatic withdrawal behaviors in placebo-implanted control rats \( F_{(1,21)} = 2.395, \text{NS} \). However, the lowest dose of naloxone tested \(0.01 \text{ mg/kg} \) failed to induce somatic withdrawal signs in morphine-dependent rats that were significantly greater than those observed in placebo-implanted rats also treated with naloxone \(0.01 \text{ mg/kg} \).

Aversive states are associated with P-CREB induction in the NAc

To identify molecular mechanisms that might underlie morphine withdrawal-induced aversive states, we performed a dose-effect function of naloxone on P-CREB and P-GluR1 induction in the NAc and CPU. The effects of naloxone on P-CREB depended on dose \( \text{NAc: } F_{(3,16)} = 8.621, p < 0.01; \text{CPU: } F_{(3,16)} = 5.219, p < 0.05 \). In both brain regions, levels of P-CREB in morphine-dependent rats treated with naloxone \(1.0 \text{ mg/kg} \) compared with placebo control rats treated with naloxone \(1.0 \text{ mg/kg} \) were significantly greater than levels of P-CREB in morphine-dependent rats treated with vehicle \(0.0 \text{ mg/kg} \) compared with placebo control rats treated with vehicle (Fig. 2A,B). Because naloxone \(1.0 \text{ mg/kg} \) induces place aversions in both morphine-
dependent and nondependent rats (Fig. 1A), it is possible that this dose of naloxone could affect P-CREB expression in placebo control rats as well as morphine-dependent rats. Absolute fold inductions (±SEM) of P-CREB in the NAc and CPU of rats treated with naloxone (1.0 mg/kg) compared with placebo controls treated with vehicle are as follows: NAc (placebo, 0.891 ± 0.118; morphine, 1.420 ± 0.173); CPU (placebo, 0.686 ± 0.109; morphine, 1.317 ± 0.324). Levels of CREB protein were unchanged in the NAc and CPU of morphine-dependent rats compared with placebo controls (fold induction in NAc: placebo, 1.001 ± 0.012 SEM; morphine, 1.07 ± 0.061 SEM, p = 0.29; fold induction in CPU: placebo, 0.999 ± 0.022 SEM; morphine, 0.965 ± 0.101 SEM, p = 0.74, Student’s t tests, seven rats per group). Naloxone failed to change levels of P-GluR1 in either the NAc (F(2,26) = 1.241, NS) or the CPU (F(2,26) = 0.631, NS) (Fig. 2C,D). Absolute fold inductions (±SEM) of P-GluR1 in the NAc and CPU of rats treated with naloxone (1.0 mg/kg) compared with placebo controls treated with vehicle are as follows: NAc (placebo, 1.115 ± 0.113; morphine, 1.405 ± 0.202) and CPU (placebo, 1.003 ± 0.125; morphine, 1.317 ± 0.324). Levels of GluR1 protein were also unchanged in morphine-dependent compared with placebo controls (fold induction in NAc: placebo, 1.00 ± 0.006 SEM; morphine, 0.983 ± 0.074 SEM, p = 0.82; fold induction in CPU: placebo, 1.00 ± 0.01 SEM; morphine, 1.013 ± 0.103 SEM, p = 0.91, Student’s t tests, eight rats per group).

To test whether the induction of P-CREB in the NAc and CPU of morphine-dependent rats by naloxone (1.0 mg/kg) is attributable to an induction of aversive-like states, as opposed to alterations in locomotor activity or other nonspecific behaviors, separate rats were treated with a dose of LiCl (120 mg/kg) known to cause dysphoria (Shippenberg et al., 1988; Tomaszewicz et al., 2006). LiCl significantly increased P-CREB in the NAc (p < 0.001, Student’s t test) (Fig. 2A, inset) but not the CPU (Fig. 2B, inset). Likewise, LiCl did not affect P-GluR1 levels in either the NAc or CPU (Fig. 2C,D, insets).

**Systemic administration of a dopamine D1 receptor agonist is rewarding in morphine-dependent rats**

To determine whether morphine dependence alters the motivational impact of dopamine D1 receptor stimulation, rats with subcutaneous implants of either morphine or placebo pellets were treated with the D1 receptor agonist SKF 82958 and tested in the place conditioning paradigm. The doses of SKF 82958 that we chose were based on previous behavioral studies demonstrating that D1 agonists prevent cocaine-induced reinstatement of cocaine-seeking behavior (Self et al., 1996b). The effects of SKF 82958 depended on conditioning (F(1,12) = 11.778; p < 0.01) (Fig. 3A). Simple main effects tests reveal that SKF 82958 (1 mg/kg) was only rewarding in morphine-dependent rats (F(1,12) = 12.113; p < 0.01).

**Dopamine D1 receptor agonists block affective and somatic signs of morphine withdrawal**

To test the hypothesis that activation of D1 receptors during morphine withdrawal would exacerbate signs of morphine withdrawal, morphine-dependent rats were treated with naloxone plus SKF 82958. A low dose of naloxone (0.01 mg/kg) was used to selectively precipitate affective signs of morphine withdrawal, which were measured using place conditioning. Naloxone failed to induce place aversions in morphine-dependent rats in the presence of any dose of SKF 82958 (0.1, 0.3, 1.0 mg/kg) (Fig. 3B). Furthermore, SKF 82958 dose-dependently elicited place preferences in the presence of naloxone (0.01 mg/kg) in morphine-dependent rats (F(2,43) = 3.662; p < 0.05). Simple main effects tests reveal that SKF 82958 (1.0 mg/kg) was rewarding in morphine-dependent rats treated with naloxone (F(1,17) = 13.329; p < 0.05). For reference, the effects of naloxone (0.01 mg/kg) alone on place conditioning are shown (see "0" dose of D1 agonist in Fig. 3B).

To determine whether activation of D1 receptors could also affect somatic signs of morphine withdrawal, rats were coadministered a moderate dose of naloxone (0.1 mg/kg) shown to induce both place aversions and somatic withdrawal signs (Fig. 1A,B) and SKF 82958 (1.0 mg/kg). The effects of SKF 82958 on the sum of all naloxone-induced somatic signs depended on an interaction between treatment and dependence (F(2,28) = 17.315; p < 0.0001) (Fig. 4A). Simple main effects tests reveal that treatment did not effect total somatic withdrawal behaviors in placebo-implanted control rats (F(2,14) = 1.273, NS) but did have a significant effect on behavior in morphine-dependent rats (F(2,14) = 79.754; p < 0.0001). SKF 82958 significantly reduced the expression of somatic withdrawal behaviors compared with naloxone alone, but rats treated with naloxone and SKF 82958 still displayed more withdrawal behaviors than rats treated with SKF 82958 alone. To further examine which specific somatic withdrawal behaviors were affected by activation of D1 receptors, we analyzed wet dog shakes, teeth chattering, and cage crossings (Fig. 4B–D). Wet dog shakes and teeth chattering are quite specific for opiate withdrawal, whereas cage crossing is a more general locomotor effect. We found that the effects of SKF 82958 on wet dog shakes, teeth chattering, and cage crossings depended on interactions between treatment and dependence (F(2,28) = 9.865, p < 0.001; F(2,28) = 13.523, p < 0.0001; F(2,28) = 3.728, p < 0.05, respectively). Simple main effects tests reveal that treatment did not effect any behavior in placebo-implanted rats, whereas SKF 82958 significantly reduced the expression of naloxone-induced wet dog shakes and teeth chattering. Naloxone-induced cage crossings in morphine-dependent rats were not affected by SKF 82958.

D1 receptor-mediated induction of P-GluR1 in the NAc of morphine-dependent rats is attenuated by naloxone

To test whether the behavioral effects of SKF 82958 in morphine-dependent rats are correlated with changes in the function of
CREB and GluR1 in the NAc and CPu, levels of P-CREB and P-GluR1 were measured in response to systemic administration of the D1 agonist. Interestingly, SKF 82958 failed to significantly increase P-CREB in either the NAc (F(5,48) = 0.328, NS) or CPu (F(5,47) = 1.207, NS) under any condition tested (Fig. 5A,B). In contrast, SKF 82958 significantly increased P-GluR1 levels in both the NAc (F(5,51) = 14.786; p < 0.0001) and CPu (F(5,51) = 3.883; p < 0.01) (Fig. 5C,D). Post hoc analysis showed that, in the NAc, SKF 82958 significantly induced P-GluR1 in all treatment groups compared with P-GluR1 levels in placebo-implanted rats treated with vehicle. Interestingly, naloxone decreased SKF 82958-mediated P-GluR1 expression in morphine-dependent rats. Hence, P-GluR1 levels in morphine-dependent rats treated with naloxone and SKF 82958 were not significantly different from P-GluR1 levels in morphine-dependent rats treated with naloxone alone. In the CPu, SKF 82958 significantly increased P-GluR1 levels only in nondependent (placebo) rats.

Discussion

“Unmasking” of opiate-induced increases in cAMP-mediated signaling likely contributes to behavioral signs of withdrawal (Nestler and Aghajanian, 1997). The current studies were designed to examine whether two targets of cAMP signaling, CREB and GluR1, would be activated in response to withdrawal-associated aversive states and whether activation of D1 receptors would potentiate cAMP signaling in the NAc and exacerbate withdrawal-associated aversive states. Previous work has demonstrated that activation of CREB and GluR1 within the NAc contributes to aversive effects (Carlezon et al., 1998; Kelz et al., 1999; Pliakas et al., 2001). We found that P-CREB, but not P-GluR1, was increased in morphine-dependent rats treated with a high dose of naloxone that elicits both motivational and somatic abstinence signs of withdrawal. Unexpectedly, systemic administration of the DA D1 agonist SKF 82958 reduced motivational and somatic signs of morphine withdrawal and was rewarding on its own in morphine-dependent rats, although it had no behavioral effects in nondependent rats. Furthermore, SKF 82958 increased P-GluR1, but not P-CREB, in the NAc. However, SKF 82958-stimulated P-GluR1 expression was decreased in morphine-dependent rats cotreated with naloxone. These findings suggest that the behavioral and molecular consequences of D1 receptor activation are altered by morphine dependence.

Dissociation of motivational and somatic withdrawal signs

We dissociated motivational and somatic signs of morphine dependence by precipitating withdrawal with several naloxone doses, similar to previous work (Schulteis et al., 1994). A low dose of naloxone resulted in significant place aversions with few overt signs of somatic withdrawal, whereas higher doses of naloxone elicited both place aversions and robust signs of somatic withdrawal. The ability to measure motivational signs of withdrawal independently of somatic signs allowed us to examine molecular correlates of each in the NAc and CPu, a brain region implicated in motor processing (Mink, 1996). We found that neither CREB nor GluR1 protein levels were altered in morphine-dependent rats. This is in contrast with a previous report that chronic mor-
phine decreases CREB immunoreactivity in the NAc, with no change in the CPu (Widnell et al., 1996). However, the total amount of morphine administered and the times at which CREB levels were examined differ between these studies, making comparisons difficult.

We found that a high dose of naloxone increased P-CREB levels in the NAc and CPu of morphine-dependent rats. A high dose of LiCl caused an induction of P-CREB in the NAc but not the CPu. Similar doses of LiCl produce aversive-like effects in behavioral assays including place conditioning (Shippenberg et al., 1988), conditioned taste aversion (Swank and Bernstein, 1994), and intracranial self-stimulation (Tomasiwicz et al., 2006). These findings suggest that increased CREB function in the NAc contributes to the aversive aspects of morphine withdrawal, whereas increased CREB function in the CPu might contribute to increased locomotor activity associated with withdrawal (Punch et al., 1997). This confirms and extends previous work indicating that CREB in the NAc is associated with depressive- and aversive-like states (Carlezon et al., 2005).

Despite finding that a low dose of naloxone selectively elicited motivational signs of morphine withdrawal, we did not detect a parallel induction of P-CREB or P-GluR1. This suggests that low doses of naloxone might be insufficient to activate signaling pathways required for phosphorylation of CREB and GluR1. Both PKA and glutamate-mediated increases in Ca$^{2+}$ are necessary for CREB activation, but only PKA can induce GluR1 phosphorylation at Ser$^{845}$ (Sheng et al., 1991; Chao et al., 2002a). Thus, naloxone-induced P-CREB in morphine-dependent rats might reflect synergism between signaling from withdrawal-induced glutamate release (Sepulveda et al., 1998) and increased cAMP. Other studies have shown that low doses of naloxone induce c-fos and Fos-related antigens (FRAs) in the NAc of morphine-dependent rats (Walters et al., 2000; Gracy et al., 2001). Fos can be activated by Ca$^{2+}$-mediated signaling and is not necessarily reliant on P-CREB (Lerea and McNamara, 1993), suggesting different sensitivities and functions for these various proteins.

**Dependence-associated effects of D$_1$ receptor activation**

The D$_1$ receptor agonist SKF 82958 elicited place preferences in morphine-dependent, but not nondependent, rats. Other studies have shown that the effects of D$_1$ receptor agonists on place conditioning in drug-naive rats are variable: a low dose of SKF 82958 can produce place preferences (Abrahams et al., 1998), whereas other D$_1$ agonists fail to establish place conditioning or cause place aversions (White et al., 1991). Our findings are consistent with increasing evidence that chronic drug treatment regulates the physiological and behavioral impact of D$_1$ receptor stimulation (Bonci and Williams, 1996; Laviolette et al., 2002). For example, DA does not appear to be necessary for the acute rewarding effects of morphine, but it is required for reward during withdrawal (Bechara et al., 1998; Hnasko et al., 2005) (but see Shippenberg and Herz, 1988).

SKF 82958 blocked place aversions established by a low dose of naloxone in morphine-dependent rats. Because there is some evidence that high doses of SKF 82958 can have effects at D$_2$ receptors (Ruskin et al., 1998), we tested the more selective D$_1$ agonist SKF 81297 [(±)-6-chloro-PB hydrobromide] in pilot studies and found a similar blockade of naloxone-induced place aversions in morphine-dependent rats (data not shown). SKF 82958 produces D$_1$-selective effects at doses similar to those used in this study (Self et al., 1996a), suggesting that our results are attributable primarily to D$_1$ receptor stimulation.

Rats treated with the highest dose of SKF 82958 plus naloxone exhibited place preferences equivalent to those observed with SKF 82958 alone in morphine-dependent rats. One possible explanation for this effect is that the rewarding properties of D$_1$ agonists overshadow the aversive effects of withdrawal induced by a low dose of naloxone. This seems unlikely because doses of SKF 82958 that were not rewarding on their own in morphine-dependent rats also block naloxone-induced place aversions. Also, it has been shown previously that a morphine blocks withdrawal-induced place aversions but fails to block LiCl-induced place aversions in morphine-dependent rats (Laviolette et al., 2002), suggesting that the rewarding effects of DA receptor activation do not override all aversive stimuli.

The effects of D$_1$ receptor stimulation during morphine withdrawal were not limited to attenuation of motivational signs: SKF 82958 also reduced somatic withdrawal signs. This is intriguing because previous data indicate that systemic apomorphine reduced somatic withdrawal signs, whereas intra-NAc administration of a D$_1$ agonist potentiated one somatic withdrawal sign (wet dog shakes) (Harris and Aston-Jones, 1994). We found that systemic administration of SKF 82958 blocked both wet dog shakes and teeth chattering, raising the possibility that effects of SKF 82958 on morphine withdrawal do not occur exclusively within the NAc.

**Mechanisms of D$_1$ agonist effects in morphine-dependent rats**

We examined whether D$_1$ receptor activation would exacerbate aversive states during morphine withdrawal and whether this effect would be associated with increased cAMP signaling in the NAc. Unexpectedly, SKF 82958 failed to reliably induce P-CREB under any conditions. This is in contrast to our previous findings in primary cultures of striatal neurons, in which we observed robust SKF 82958-mediated P-CREB induction during naloxone-prefactitated withdrawal from chronic morphine treatment (Chartoff et al., 2003b). Dissociated striatal cultures lack endogenous dopaminergic input; the fact that DA denervation leads to supersensitivity of D$_1$ receptor signaling (Chartoff et al., 2001) could explain the ability of SKF 82958 to induce P-CREB in striatal cultures. It is possible that SKF 82958 might activate P-CREB in vivo at higher doses or by other routes of administration (Haberny et al., 2004). Alternatively, the stress of handling and injections might have contributed to P-CREB induction in control rats (Inglis and Moghaddam, 1999), such that it was not possible to observe additional D$_1$ receptor-mediated increases in P-CREB.

In contrast, SKF 82958 significantly induced P-GluR1 in the NAc, but not the CPu, of morphine-dependent rats. GluR1 phosphorylation can increase synaptic expression of GluR1 receptors and enhance AMPA transmission, including influx of Ca$^{2+}$ (Song and Huganir, 2002; Mangiavacchi and Wolf, 2004). Interestingly, increased glutamatergic transmission and subsequent Ca$^{2+}$ influx in the NAc is generally associated with aversive states (Kelz et al., 1999; Chartoff et al., 2006). Our findings suggest that, in drug-dependent animals, the downstream consequences of increased P-GluR1 activity or AMPA receptor function are fundamentally altered. For example, the effect of increased AMPA transmission might be different in rats with dependence-associated alterations in intracellular signaling cascades that regulate the function of ion channels, which could cause dramatic alterations in the output of NAc neurons. These types of changes have been observed during cocaine withdrawal (Zhang et al., 1998) and may contribute to altered behavioral sensitivity to AMPA receptor stimulation in cocaine-experienced rats (Pierce...
et al., 1996; Cornish and Kalivas, 2000). Indeed, some aspects of stimulant-induced sensitization are correlated with increased synaptic expression of GluR1 receptors (Boudreau and Wolf, 2005).

Although the behavioral effects of SKF 82958 were the same in morphine-dependent rats and dependent rats treated with naloxone, the molecular effects differed. SKF 82958 increased P-GluR1 selectively in the NAc of morphine-dependent rats, but the D1 receptor-mediated increase in P-GluR1 was significantly reduced in dependent rats treated with naloxone. P-GluR1 is rapidly dephosphorylated during AMPA receptor activation in the NAc (Snyder et al., 2003); because glutamate release is increased in the NAc during morphine withdrawal (Sepulveda et al., 1998), a concomitant increase in AMPA receptor activation and GluR1 dephosphorylation would be expected. It is thus possible that the rewarding effects of SKF 82958 observed in morphine-dependent rats and those treated with naloxone are mediated in different brain regions. For example, during morphine withdrawal, D1 receptor stimulation in the ventral tegmental area leads to increased DA cell activity, which might alleviate withdrawal-associated aversive states (Bonci and Williams, 1996). Future studies involving brain microinjections may clarify whether D1 agonist-induced increases in P-GluR1 within the NAc are critical for establishing place preferences in morphine-dependent rats. Microinjection studies are beyond the scope of the present work because they will likely involve numerous D1 receptor-containing brain regions. Regardless, these findings suggest for the first time that D1 agonists might be useful for the treatment of withdrawal symptoms that contribute to the maintenance of opiate addiction in humans.

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