Molecular and Behavioral Interactions Between Central Melanocortins and Cocaine

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ABSTRACT

Behavioral and molecular studies have established a link between drugs of abuse and the central melanocortin system, particularly the melanocortin 4 receptor (MC4-R). The present study expands this line of investigation to characterization of the neurochemical and behavioral interactions between MC4-R and the psychomotor stimulant, cocaine. The results demonstrate that repeated, but not acute, cocaine administration up-regulates MC4-R mRNA expression in the striatum and hippocampus, but not cerebral cortex. Pharmacological studies indicate that the up-regulation of MC4-R expression occurs via dopamine D1 and D2 receptor-dependent mechanisms. The D1/D2 antagonist haloperidol and the D2-selective antagonist eticlopride mimic the effect of cocaine on MC4-R expression. In addition, coadministration of a D1-selective antagonist, SCH 23390 [R-(+)/-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine], completely blocks the up-regulation of MC4 mRNA by cocaine, demonstrating that D1 receptor activation is necessary for this response. Moreover, the results demonstrate that cocaine treatment increases behavioral responses (grooming and locomotor activity) to infusions of a melanocortin agonist, indicating that up-regulation of MC4-R expression results in functional consequences. These data further support a role for the melanocortin-MC4-R neuropeptide system in the biochemical and behavioral effects of cocaine.

Pro-opiomelanocortin (POMC) serves as a precursor for a number of neuropeptides, including the melanocortins, α-, β-, and γ-melanocyte-stimulating hormone (MSH), and adrenocorticotropic hormone, as well as β-endorphin. POMC-expressing neurons are located in the pituitary, the arcuate nucleus of the hypothalamus, and the nucleus of the solitary tract; and the latter two structures send projections to a number of different brain regions, including the mesolimbic dopamine system (Jacobowitz and O’Donohue, 1978; Eskay et al., 1979). The melanocortin neuropeptides are reported to influence a variety of behavioral and neuroendocrine systems via regulation of central nervous system centers, including grooming, thermoregulation, and learning (Alvaro et al., 1997). The actions of melanocortins are mediated via activation of melanocortin receptors, of which there are five subtypes, all of which belong to the G-protein-coupled receptor superfamily. Two of these, melanocortin-3 and -4 (MC3-R and MC4-R) are expressed in significant levels in the brain and are thereby thought to mediate the central actions of melanocortin neuropeptides.

Melanocortins are also reported to play a role in the behavioral and neurochemical actions of opiates, psychostimulants, and alcohol. Early studies indicated that melanocortin treatment could antagonize opiate tolerance and dependence, and even induce opiate withdrawal-like effects in naive animals (Szekaly et al., 1979; Contreras and Takemori, 1984). A preliminary study also found that a selective MC4-R agonist attenuates the signs of morphine withdrawal that are induced by naloxone in opiate-dependent animals (Zhou et al., 2001). We have also found that chronic administration of morphine down-regulates the expression of MC4-R in striatum and periaqueductal gray, two regions implicated in drug reward and withdrawal, providing additional support that the MC4-R subtype is involved in the long-term actions of opiates (Alvaro et al., 1996). With regard to psychostimulants, a recent study demonstrates that administration of a melanocortin agonist augments the threshold lowering effect of amphetamine for lateral hypothalamic self-stimulation (Cabeza de Vaca et al., 2002). In addition, we have recently reported that chronic cocaine treatment increases the expression of MC4-R mRNA in striatum and that administration of a melanocortin antagonist blocks the rewarding and lomomo-

ABBREVIATIONS: POMC, pro-opiomelanocortin; α-MSH, α-melanocyte-stimulating hormone; SCH 23390, R-(+)/-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; MC3-R, melanocortin 3 receptor; MC4-R, melanocortin 4 receptor; ANOVA, analysis of variance.
tor-activating effects of cocaine (Hsu et al., 2002). Finally, administration of a melanocortin receptor agonist has been shown to alter ethanol intake in alcohol-prefering rats (Ploj et al., 2000).

The current study was initiated to extend these findings in three key areas: to determine the 1) time course and 2) dopaminergic receptor subtypes that are responsible for cocaine up-regulation of MC4-R expression, and 3) the behavioral consequences of cocaine treatment on melanocortin function. The results demonstrate that repeated, but not acute, administration of cocaine up-regulates MC4-R expression in the striatum of rat brain and that this effect is dependent on D1 receptor activation. In addition, the results demonstrate that the effects of a-MSH on grooming behavior are increased by cocaine treatment. These findings provide additional support that the melanocortin-MC4-R system plays a role in the long-term actions of cocaine and morphine and begin to elucidate the neurobiological mechanisms underlying these effects.

Materials and Methods

**Drug Treatments.** All drugs were administered i.p., and rats (male Sprague-Dawley, 160–180 g) were sacrificed 3 h after the final injection. The doses and 15-day time point for cocaine and other drug treatments were chosen based on previous neurochemical studies (Hope et al., 1992; Nibuya et al., 1995; Nye et al., 1995; Horger et al., 1999): cocaine, 15 mg/kg, one single treatment (1 day) or twice daily for 4 or 14 days with a single injection following day 5 or 15, respectively; haloperidol, 2 mg/kg once daily for 21 days; eticlopride, 0.5 mg/kg once daily for 15 days; apomorphine, 1.0 mg/kg twice daily for 14 days and a single treatment on day 15; SCH 23390, 0.5 mg/kg once daily for 15 days; desipramine, 10 mg/kg twice daily for 14 days and a single treatment on day 15; fluoxetine, 3 mg/kg twice daily for 14 days and a single treatment on day 15; movepin, high dose: 75 mg subcutaneous pellet, one per day for 5 days; morphine, escalating dose: 10 mg/kg on days 1 and 2; 20 mg/kg on days 3 and 4; 40 mg/kg on days 5 and 6; 80 mg/kg on days 7 and 8; 120 mg/kg on days 9, 10, and 11; morphine, low dose: 2 mg/kg once daily for every other day for 11 days.

**RNase Protection and in Situ Hybridization Analysis.** Total RNA from various rat brain regions was extracted by homogenization in guanidine isothiocyanate followed by centrifugation through cesium chloride gradients. A 185-bp [α-32P]CTP-labeled antisense riboprobe synthesized from rat MC4-R cDNA (Alvaro et al., 1996) was hybridized at 63°C for 18 h with 20 to 30 μg total RNA per sample. Samples were RNase-treated, precipitated, resuspended in 80% formamide, and run on 8% polyacrylamide gels as described previously (Alvaro et al., 1996). Gels were dried and exposed to X-ray film with an intensifier screen, and autoradiograms were quantified using a phosphorimaging process.

In situ hybridization analysis of POMC mRNA was conducted with a 246-bp [α-32P]CTP-labeled mouse antisense riboprobe according to standard procedures (Nibuya et al., 1995). Briefly, the radio-labeled POMC riboprobe was hybridized with formaldehyde-fixed coronal sections containing the hypotalamic arcuate nucleus at 55°C for 18 h. Sections were then RNase-treated, washed in 0.1× standard saline citrate at 55°C, and exposed to X-ray film for 6 days. Images were captured on digital video and quantified using NIH Image.

**Surgery.** Bilateral indwelling cannulae (23-gauge) were implanted under Equithesin (4.5 mg/kg i.p.) anesthesia. Standard stereotaxic (David Kopf, Tujunga, CA) procedures were used with aseptic surgical techniques. Coordinates for i.c.v. infusions were based on the atlas of Paxinos and Watson (1982) with skull flat: anterior–posterior –1.3 from bregma, medial–lateral ±0.5 from the midline, dorsoventral –4.5 from skull. Stainless steel guide cannulae were lowered in pairs while mounted on “stylettes” fixed to electrode carriers. They were aimed to terminate 1.0 mm above the infusion site and were cemented in place with light curing dental resin. A stylette was used to achieve permanent guide cannulae implantation and fixation to the skull. Temporary stylettes were placed in the guide cannula to prevent occlusion. Animals were allowed a minimum of 7 days to recover, and stylettes were checked regularly.

**Drug Microinfusions.** Infusions (i.c.v.) of α-MSH (1.0 μg/1.0 μl; Sigma-Aldrich, St. Louis, MO) were made bilaterally 15 min before the test. Injection cannulae (31-gauge), attached to PE-10 polyethylene tubing (Clay Adams, Parsippany, NJ) were manually lowered through the guides to the final infusion site. A microdrive pump (Harvard Apparatus, South Natick, MA) with 10-μl Hamilton microsyringes (Hamilton Company, Reno, NV) was used to deliver the drug. All infusions were made bilaterally in volumes of 0.5 μl over a period of 2 min with an additional 2 min allowed to elapse prior to removal of the infusion needles and replacement of the stylettes. Subjects were hand-held during the infusions.

**Behavioral Analysis.** Before the drug treatment phase, animals were habituated to the locomotor activity boxes for 1 h over two sessions. One week after surgery to implant cannula (i.c.v.), subjects were administered saline or cocaine (15 mg/kg, twice daily for 14 days).

Three hours after the last cocaine or saline treatment, subjects were given bilateral infusions of α-MSH or saline i.c.v. Five minutes later, they were placed in the activity monitoring cages, and locomotor behavior was measured for 60 min. Locomotor activity was quantified using the automated Omnitech (Columbus, OH) Digiscan Micro-monitor system equipped with 16 photocells per chamber. Locomotor activity or ambulation, defined as consecutive beam breaks, was collected in 10-min intervals. The activity chambers were located inside a sound-attenuated room equipped with a white noise generator. During activity testing, the room was illuminated with red light only. Forty minutes after the infusions, subjects were also assessed for several other behaviors (i.e., time spent grooming, number of grooming bouts, stretching, and rearing) by an observer unaware of the treatment. Grooming activity did not result in consecutive beam breaks and therefore did not interfere with locomotor activity measurements. These assessments were made over a 10-min period. Significant behavioral effects were further analyzed by factorial ANOVA with Scheffé’s P test. Data are expressed as mean ± S.E.M.

**Results**

**Time Course for the Induction of MC4-R mRNA by Cocaine Treatment.** In a previous study we have found that chronic administration of morphine significantly decreases the expression of MC4-R mRNA in the striatum of rat brain (Alvaro et al., 1996). To extend these findings to another drug of abuse, the time course for the influence of repeated cocaine administration on the expression of MC4-R in rat brain was determined. Rats received a single dose of cocaine (1 day) or were administered cocaine twice daily for 4 or 14 days, and on the next morning (5th or 15th days), a final cocaine injection was given. In all cases tissue was harvested 3 h later. Levels of MC4-R mRNA were determined by RNase protection analysis using a radiolabeled antisense MC4-R riboprobe (Fig. 1). In our previous study we reported that only MC4-R, and not MC1-R or MC3-R, was expressed in most brain regions (MC3-R is expressed in hypothalamus and periaqueductal gray, but not striatum) (Alvaro et al., 1996). In the present study we found that repeated cocaine administration resulted in a highly significant and robust up-regulation of MC4-R mRNA in striatum and hippocampus.
The up-regulation of MC4-R mRNA was dependent on repeated cocaine exposure, as there was no significant effect at the earlier time points examined (1 and 4 days of treatment) (Fig. 2). 

Pharmacological Characterization of the Induction of MC4-R by Cocaine. Studies were next conducted to characterize the dopamine receptor subtypes that account for up-regulation of MC4-R mRNA by repeated cocaine. The actions of cocaine on the mesolimbic dopamine system involve regulation of D1 and D2 receptors, albeit an indirect effect via inhibition of dopamine reuptake (Wise, 1996; White and Kalivas, 1998). The influence of a number of direct-acting D1 and D2 antagonists and agonists on the up-regulation of MC4-R was examined to determine whether increased expression occurs through activation of a specific dopamine receptor subtype. The drug doses were chosen based on a previous study (Nye et al., 1995). Chronic administration of the nonselective D1/D2 antagonist haloperidol, like cocaine, also resulted in a significant up-regulation of MC4-R mRNA in striatum (Fig. 3). Similar results were seen following chronic administration of a D2-selective antagonist, eticlopride. This effect was not observed with chronic administration of either a D1-selective antagonist, SCH 23390, or a nonselective D1/D2 agonist, apomorphine. The effects of two antidepressant drugs that do not directly influence dopamine reuptake or transmission were also examined. Repeated administration of desipramine, a norepinephrine selective reuptake inhibitor, or flouxetine, a serotonin selective reuptake inhibitor, did not significantly alter levels of MC4-R mRNA in the striatum.

Previous studies have reported that the actions of cocaine on c-Fos are dependent on activation of dopamine D1 receptors in striatum, even though D1 receptor activation alone is not sufficient to mimic the effect of cocaine (Graybiel et al., 1990; Young et al., 1991; Couceyro et al., 1994; Nye et al., 1995). To determine whether regulation of MC4-R mRNA is dependent on activation of D1 receptors, a study was undertaken to determine whether inhibition of this receptor could alter cocaine up-regulation of MC4-R. For this study, animals were pretreated (30 min) with either saline or a D1 antagonist, SCH 23390, and then received cocaine twice daily for 15 days. Co-administration of SCH 23390 completely blocked the up-regulation of MC4-R mRNA that is observed after cocaine administration alone (Fig. 4).

Regulation of MC4-R Expression by Repeated Morphine Administration. In a previous study we found that chronic administration of morphine decreased the expression of MC4-R mRNA in striatum (Alvaro et al., 1996). Given this finding, the results of the current investigation demonstrating that cocaine administration increases MC4-R expression were surprising. However, the dose of morphine used in the previous study was very high (75-mg pellet once a day) and the length of treatment was relatively short (5 days). This treatment regimen is often used to rapidly produce a state of...
tolerance and dependence (Reith et al., 1987; Rasmussen et al., 1990). In addition, the dose is much higher and the treatment time shorter than those that are used to produce behavioral sensitization to morphine (2 mg/kg per day for 10 days) and that would produce a behavioral state more comparable with that produced by the cocaine sensitization regimen used in the current study (Babbini and Davis, 1972; Reith et al., 1987). Therefore, a study was conducted to determine the influence of a low dose of morphine on the expression of MC4-R mRNA.

Administration of morphine using the low-dose (2 mg/kg, 10 days) paradigm significantly increased the expression of MC4-R mRNA in striatum, similar to the effect of cocaine (Fig. 5). In contrast, the high-dose morphine regimen produced a significant down-regulation of MC4-R mRNA as reported in our previous study (Fig. 5). An intermediate regimen was also tested, in which the dose of morphine is increased during the course of treatment. Using this intermediate, escalating morphine dose regimen, the expression of MC4-R was not significantly altered, presumably because of the counteracting effects of the tolerance and sensitization effects produced by this treatment paradigm.

**Fig. 3.** Influence of pharmacological agents on MC4-R mRNA in striatum. Rats were administered saline or one of the following psychotropic drugs: cocaine (15 mg/kg, twice daily for 14 days and a single treatment on day 15), a nonselective D1/D2 receptor antagonist (haloperidol, 2.0 mg/kg, once daily for 14 days and a single treatment on day 15), a nonselective D1/D2 receptor agonist (apomorphine, 1.0 mg/kg, twice daily for 14 days and a single treatment on day 15), a selective D1 receptor antagonist (eticlopride, 0.5 mg/kg, once daily for 14 days and a single treatment on day 15), a norepinephrine selective reuptake blocker (desipramine, 10 mg/kg, twice daily for 14 days and a single treatment on day 15), or a 5-HT selective reuptake inhibitor (fluoxetine, 3 mg/kg, twice daily for 14 days and a single treatment on day 15). Tissue was harvested 3 h after the last drug treatment, and levels of MC4-R mRNA were determined by RNase protection analysis and quantified by densitometry. The results are expressed as percentage of sham-treated controls and are the mean ± S.E.M. (n = 6 per group). *, p < 0.05; **, p < 0.005, compared with saline-treated controls (Student’s t test).

**Fig. 4.** Cocaine induction of MC4-R mRNA is blocked by pretreatment with a D1 receptor antagonist. Rats were administered SCH 23390, 30 min before cocaine administration at each of two treatment times each day for 14 days and once on day 15. Levels of MC4-R mRNA in striatum were determined by RNase protection analysis and were quantified by densitometry. Results are expressed as percentage of respective sham-treated controls and are the mean ± S.E.M. (n = 6 per group). *, p < 0.05 with respect to control and to cocaine + SCH 23390 animals (ANOVA and Fisher’s post hoc test).

**Fig. 5.** Influence of different morphine treatment regimens on MC4-R mRNA expression in striatum. Rats were administered one of three different dose and time regimens: a high dose of morphine via 75-mg subcutaneous pellets for 5 days (HIGH), an intermediate, escalating dosing schedule (10 mg/kg on days 1 and 2; 20 mg/kg on days 3 and 4; 40 mg/kg on days 5 and 6; 80 mg/kg on days 7 and 8; 120 mg/kg on days 9–11, all i.p. once daily) (ESCALATING), or a low dose of 2 mg/kg (i.p.) every other day for 10 days (LOW). Three hours after the last morphine treatment, levels of MC4-R mRNA were determined by RNase protection analysis. Results are expressed as percentage of respective sham-treated controls and are the mean ± S.E.M. (n = 6 per group). *, p < 0.05 with respect to controls (Student’s t test).
These results indicate that the regulation of MC4-R by morphine correlates with the behavioral effects of the different treatment regimens. However, it is also possible that the different treatment protocols (i.e., s.c. morphine pellets under halothane anesthesia versus i.p. morphine injections) result in altered control levels of MC4-R that could influence the effect of morphine treatment.

**Influence of Cocaine Administration on the Expression of POMC mRNA in Arcuate Nucleus.** To provide a complete examination of the effects of cocaine on the melanocortin system, the expression of POMC mRNA in the hypothalamus was also studied. Levels of POMC mRNA in the arcuate nucleus of hypothalamus were determined by in situ hybridization analysis using an antisense mouse POMC riboprobe.Repeated administration of cocaine, using the regimen described above for the MC4-R mRNA studies, resulted in a significant down-regulation of POMC mRNA levels in the hypothalamic arcuate nucleus (Fig. 6).

**Influence of Cocaine Administration on Grooming Behavior and Locomotor Activity.** To determine whether the up-regulation of MC4-R results in alteration of melanocortin receptor function, a well established behavioral response to melanocortins, grooming, as well as locomotor activity, was examined. Grooming is one of the most robust acute actions of melanocortins (Gispen et al., 1975), and increased grooming is mediated by activation of MC4-R (Von Frijtag et al., 1998; Adan et al., 1999). The amount of time spent grooming and the number of grooming bouts, as well as the time spent stretching and rearing, were determined by an observer blinded to the treatment condition. Animals were implanted with bilateral cannulae into the lateral ventricles and, after a short recovery time, were treated with cocaine for 14 days. Three hours after the final injection of cocaine, the animals received bilateral i.c.v. infusion of α-MSH or saline. The 3-h time point was chosen because the locomotor activating effects of cocaine are no longer apparent and therefore would not compete with other behavioral responses (Post and Rose, 1976) (also see below). Forty minutes after the infusion, grooming behavior was measured over a 10-min period. Bilateral saline infusions (i.c.v.) resulted in very little or no grooming behavior in animals previously treated with either saline or cocaine (Fig. 7). In contrast, bilateral α-MSH infusions (i.c.v.) produced a robust induction of grooming in the saline-treated control animals, and this effect was significantly increased by approximately 2-fold in animals that received the chronic cocaine treatment regimen (Fig. 7).

Although melanocortin administration has not been reported to influence basal locomotor activity per se (Isaacson and Green, 1978; Van Erp et al., 1991), this measure was also examined. Locomotor activity was examined in the same animals analyzed for grooming and according to the same treatment protocol: animals were treated chronically with either cocaine or saline, and 3 h later, they received i.c.v. infusions of either α-MSH or saline and were placed in locomotor activity chambers. A moderate level of activity was observed in all groups at the first time point, most likely as a result of handling and introduction into the activity chambers. There was no significant elevation of locomotor activity in the cocaine-treated groups at the first 10-min time interval as expected, because the locomotor activation in response to

![Fig. 6.](image)

**Fig. 6.** Chronic cocaine treatment increases the expression of POMC in arcuate nucleus. Rats were treated with cocaine or saline twice daily for 14 days and with a final injection on the morning of day 15 as described above. Three hours later, the brains were harvested for in situ hybridization analysis of POMC mRNA in hypothalamus using a 35S-labeled riboprobe. Representative autoradiograms are shown for three brain regions, and levels of POMC mRNA were quantified by densitometry. The results are expressed as percentage of control and are the means ± S.E.M. (n = 6 per group). *, p < 0.05 with respect to controls (Student’s t test).

![Fig. 7.](image)

**Fig. 7.** Effect of chronic cocaine administration on α-MSH-induced grooming behavior. Rats were treated chronically with saline (sal) or cocaine (coc) (15 mg/kg, twice daily for 10 days) and 3 h after the last injection received bilateral i.c.v. infusions of either sal or α-MSH as indicated. Grooming behavior was then measured 40 min after infusion for a total of 10 min. The results are expressed as a percentage of control and are the means ± S.E.M. (n = 6 per group). *, p < 0.05 with respect to coc-sal and sal-sal; **, p < 0.05 with respect to sal-MSH as well as the other two groups (ANOVA and Fisher’s post hoc test).
throughout the test period, the cocaine/control groups (Fig. 8). At each subsequent 10-min interval greater than that seen in the cocaine alone group or the other ized a level of locomotor activity that was significantly

The results also demonstrate that the induction of MC4-R mRNA with respect to controls is a degree of change rarely

**Discussion**

The results of this study extend previous findings and provide further evidence of interactions between the melano-
cortin system and two drugs of abuse, cocaine and morphine. In the present study evidence is presented to demonstrate that repeated, but not acute, cocaine administration increases MC4-R expression in the striatum and decreases POMC expression in the arcuate nucleus of hypothalamus. In addition, the results demonstrate that repeated administration of cocaine augments the behavioral actions of the melano-
cortin-MC4-R neuropeptide system.

Previous work has demonstrated that only MC4-R mRNA is expressed in the striatum and that repeated administration of a high dose of morphine down-regulates MC4-R mRNA levels in this brain region (Alvaro et al., 1996). The results of the current investigation demonstrate that repeated administration of cocaine up-regulates the expression of MC4-R in the striatum and hippocampus, but not in the cerebral cortex. In the striatum, the induction of MC4-R is particularly dramatic. The 2- to 3-fold increase in MC4-R mRNA with respect to controls is a degree of change rarely reported in the literature for regulation of a neurotransmitter or neuropeptide receptor in the nervous system. The results also demonstrate that the induction of MC4-R mRNA is time-dependent in that long-term (15-day), but not acute or short-term (1- or 5-day), administration up-regulates MC4-R mRNA in striatum and hippocampus.

To examine the dopamine receptor mechanisms through which cocaine could effect such a large change in the striatum, the influence of various dopaminergic compounds on the expression of MC4-R was examined. The role of dopamine D1 and D2-like receptors in the neurochemical and behavioral actions of cocaine is complex, and both receptor subtypes are reported to be involved (Wise, 1996; White and Kalivas, 1998; White et al., 1998). The results of the present study are consistent with previous pharmacological studies and provide evidence for two major points to be made regarding the dopamine receptor subtypes that underlie cocaine regulation of MC4-R. First, the actions of cocaine on MC4-R expression are dependent on D1 receptor activation, inasmuch as daily pretreatment with a D1 receptor antagonist, SCH 23390, completely blocks the up-regulation of MC4-R mRNA. A similar pharmacological profile for D2 receptors has also been reported for cocaine reward (Maldonado et al., 1993; McGregor and Roberts, 1995) and the acute neurochemical actions of cocaine (Graybiel et al., 1990; Young et al., 1991; Couceyro et al., 1994). Second, chronic D2 receptor blockade, either with a nonselective dopamine D2/D3 receptor antagonist, haloperidol, or a very selective D2 receptor antagonist, eticlo-

pride, also increased the expression of MC4-R mRNA in striatum. D2 receptor blockade by haloperidol or eticlopride could result in up-regulation of MC4-R mRNA via blockade of autoreceptors leading to increased dopamine release (Nye et al., 1995; Rouge-Pont et al., 2002). However, it is important to point out that only single drug doses were tested, and that in some cases the doses used could be high enough to influence other neurotransmitter systems that could contribute to the up-regulation of MC4-R. For example, the doses of halo-

peridol and SCH 23390 could influence serotonin receptor subtypes (Bischoff et al., 1986; Schotte et al., 1993).

The influence of a nonselective D1/D2 receptor agonist,
apomorphine, on expression of MC4-R was also examined. Chronic administration of apomorphine produced a small induction (approximately 25%) of MC4-R mRNA that was not significant, and not nearly as efficacious as either cocaine or D2 receptor blockade. One explanation could be that the dose and pharmacokinetics of the drug are critical to the response, and that alternate treatment schedules might produce effects similar to those of cocaine. This possibility is supported by the opposite actions of two different morphine treatment regimens on the expression of MC4-R in striatum. Administration of selective D1 or D2 agonists, or combinations of these selective agonists, can mimic the acute and chronic actions of cocaine (Wise, 1996; White and Kalivas, 1998), and future studies will be needed to further examine the influence of these agents on the expression of MC4-R.

The results also demonstrate that chronic elevation of two other monoamines, norepinephrine or serotonin, via administration of selective reuptake inhibitors, does not increase the expression of MC4-R mRNA in the striatum. These results indicate that the up-regulation of MC4-R is dependent on activation of the dopamine neurotransmitter system, although it is possible that higher doses of the reuptake blockers could also result in regulation of MC4-R expression. However, as discussed for apomorphine and morphine, it is also possible that other treatment regimens could result in up-regulation of MC4-R expression in striatum. The receptor mechanisms underlying the up-regulation of MC4-R mRNA in the hippocampus were not examined but could involve some of the same dopamine receptor mechanisms that account for increased expression of MC4-R in striatum, or could result from recruitment of other neurotransmitter systems.

The pharmacological profile of MC4-R up-regulation in the striatum by dopaminergic compounds is strikingly similar to the acute and chronic regulation of c-Fos and Fos-related antigens in this brain region (Graybiel et al., 1990; Young et al., 1991; Couceyro et al., 1994; Nye et al., 1995; Hiroi et al., 1997). Chronic, but not acute, administration of morphine up-regulates the expression of ΔFosB and related isoforms in the striatum. Activation of D1 receptors is necessary for increased expression of c-Fos and ΔFosB, and D2 receptor blockade mimics the effect of cocaine. The similar time course and pharmacological profile for the induction of both ΔFosB immunoreactivity and MC4-R mRNA in the striatum is correlative but suggests that the dopamine receptor mechanisms underlying their regulation may be related. In addition, these results raise the possibility that induction of MC4-R mRNA could be mediated by up-regulation of c-Fos and ΔFosB. Studies are currently being conducted to determine whether the expression of MC4-R is altered in ΔFosB transgenic mice that exhibit enhanced sensitivity to cocaine (Kelz et al., 1999).

In a previous study, we established that chronic administration of morphine down-regulates MC4-R mRNA in the striatum (Alvaro et al., 1996), yet in the current investigation, we report an up-regulation of the MC4-R mRNA following chronic cocaine treatment. It is important to note, however, that in the previous study, chronic morphine treatment involved implanting rats with 75-mg subcutaneous morphine pellets once daily for 5 days. In other words, the dosing was both continuous and extremely high. In the present study, on the other hand, the dose of cocaine used was comparatively much lower and was also intermittent. Previous studies demonstrate that the behavioral and biochemical effects of drugs of abuse depend on the dose administered and the dosing paradigm used (Reith et al., 1987; Nye et al., 1995). Based on these findings, we hypothesized that the difference between the effect of morphine observed previously and the actions of cocaine in the current study might result from disparities in drug dosing and scheduling. Therefore, the continuous high-dose morphine paradigm used in the previous study was compared with an intermittent low-dose treatment schedule, as well as an escalating treatment regimen. As the results demonstrate, the intermittent, low-dose morphine regimen significantly up-regulates MC4-R expression in the striatum, similar to the effect of cocaine. What makes these findings particularly interesting is that MC4-R mRNA levels appear to correlate with the behavioral effects of morphine. Administration of a high dose of morphine using a continuous treatment regimen, as used in our previous study (Alvaro et al., 1996) leads to tolerance and dependence (Rasmussen et al., 1990) and decreases the expression of MC4-R in striatum. In contrast, administration of a low dose of morphine using an intermittent regimen, as used in the current study, produces behavioral sensitization (Reith et al., 1987) and increases the expression of MC4-R mRNA in striatum. This is consistent with the effects of the cocaine regimen that also produces locomotor sensitization (Horger et al., 1999) and up-regulates MC4-R expression. The results of these studies suggest that MC4-R expression can be up- or down-regulated depending on the type of drug treatment regimen used and that this may result in different behavioral responses mediated by the melanocortin-MC4-R system.

To begin to examine the behavioral interactions between the melanocortin system and repeated administration of cocaine, a melanocortin-regulated behavior was examined. The ability of α-MSH to induce excessive grooming is a well known and easily identified behavioral effect of melanocortin action in the brain (Gispen et al., 1975). The significance of these results resides in the fact that animals that receive both chronic cocaine treatment and α-MSH infusion exhibit markedly enhanced grooming relative to non-cocaine-treated controls receiving the same dose of α-MSH. Structure-activity relationship studies of melanocortin analogs indicate that grooming induction occurs primarily via activation of MC4-R and not at other MC-R subtypes (Von Frijtag et al., 1998; Adan et al., 1999). It is therefore reasonable to conclude that the increase in excessive grooming seen in chronic cocaine-treated rats results from functional up-regulation of MC4-R. At issue is in which brain region this functional up-regulation is occurring. The results of the current study implicate the striatum, which includes the dorsal striatum and nucleus accumbens. Previous studies indicate that dorsal striatum does not mediate melanocortin induction of grooming (Wiegant et al., 1977; Argiolas et al., 2000), but melanocortin infusion into the nucleus accumbens is reported to increase grooming (Ryan and Isaacson, 1983). It is also possible that regulation of MC4-R expression in other brain regions implicated in grooming behavior, including ventral tegmentum (Torre and Celis, 1988), periaqueductal gray (Spruijt et al., 1986), and paraventricular hypothalamus (Van Erp et al., 1991), also contributes to the induction of grooming observed in the present study.

The influence of melanocortin infusions and cocaine treatment on locomotor activity was also examined. α-MSH infu-
sions into the brains of cocaine-treated rats induced a significant increase in locomotor activity, and this activity was maintained throughout a 1-h test period. Although cocaine alone is known to induce substantial locomotor activity immediately after administration (Post and Rose, 1976; Roy et al., 1978), we tested animals 3 h after drug treatment, when the locomotor-activating effects are no longer exhibited (Post and Rose, 1976). The results of the current investigation are consistent with previous reports and demonstrate that animals receiving repeated cocaine and then receiving an acute saline infusion (i.c.v.) 3 h later do not exhibit locomotor activity significantly different from the saline or α-MSH alone control groups. α-MSH itself has been reported to induce very modest increases in locomotor activity, primarily in hipping and rearing (Van Erp et al., 1991). Most studies, however, do not report any significant locomotor-activating effects of α-MSH. In fact there is a report that α-MSH infusion decreases locomotor activity, possibly as a result of excessive grooming in these animals (Isaacson and Green, 1978). The results presented here on the combined effects of cocaine and α-MSH are interesting, and indicate a synergistic interaction that results in prolonged increases of locomotor activation. The molecular and cellular mechanisms underlying this interaction are not clear but could result from regulation of intracellular signaling pathways in dopaminergic target neurons in the striatum. MC4-R receptors, like D1 receptors, are positively coupled to the cAMP pathway and mediate acute effects of cocaine. Soc Neurosci 27:440–446.


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