Regulation of Opioid Receptors by Cocaine

ELLEN M. UNTERWALD

Department of Pharmacology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140, USA and

Biology of Addictive Diseases Laboratory, The Rockefeller University, New York, New York 10021, USA

ABSTRACT: Cocaine is a widely abused psychostimulant. Its direct actions include inhibition of dopamine, serotonin, and norepinephrine reuptake into presynaptic nerve terminals, thereby potentiating the actions of these transmitters in the synapse. A variety of studies have demonstrated that cocaine can also have profound effects on the endogenous opioid system. Compelling evidence points to the importance of mu opioid receptors in human cocaine addiction and craving. Animal studies support these findings and demonstrate that chronic cocaine administration can result in alterations in opioid receptor expression and function as measured by changes in critical signal transduction pathways. This chapter reviews studies on the regulation of opioid receptors as the result of exposure to cocaine.

KEYWORDS: Adenylyl cyclase; cAMP; Cocaine; Opioid receptors

INTRODUCTION

Cocaine is a widely abused psychostimulant and, as such, its pharmacological, neurochemical, and behavioral effects have been well studied. The reinforcing effects of cocaine are thought to be largely due to its ability to bind to the dopamine transporter and inhibit the reuptake of dopamine into presynaptic nerve terminals.[1–4] Cocaine also inhibits the reuptake of serotonin and norepinephrine.[2] Recent studies utilizing mice that lack the dopamine transporter[5,6] or the serotonin 1B receptor[7] have emphasized the importance of the serotonin system in the response to psychostimulants. Since reuptake is the main mechanism by which these transmitters are removed from the extracellular space and their sites of action,[8] inhibition of reuptake by cocaine

Corresponding address: Ellen M. Unterwald, Ph.D., Department of Pharmacology, Temple University School of Medicine, 3420 N. Broad Street, Philadelphia, PA 19140. Voice: 215-707-1681; fax: 215-707-1618.
eunterwa@unix.temple.edu

causes these three neurotransmitters to accumulate in the synaptic space and increases the activation of their respective receptors.[1,9,10] Cocaine, like other local anesthetic agents, also blocks voltage-gated sodium channels, but this action of cocaine is thought to be unrelated to its abuse liability.[11]

The mesolimbic dopaminergic system is believed to play a primary role in mediating the reinforcing properties of many, if not all, drugs of abuse including both cocaine and opiates.[12,13] The actions of cocaine on dopaminergic projections to the nucleus accumbens appear necessary for cocaine reward.[14] The medial prefrontal cortex,[15] ventral pallidum,[16] and olfactory tubercle[17] have also been implicated in cocaine reinforcement.

Dopaminergic neurons interact with the endogenous opioid system, and together they collectively modulate motivated and emotional behaviors and locomotor activity.[18,19] The interaction of these two systems has been noted at anatomical, neurochemical, and behavioral levels. Significant concentrations of opioid peptides and opioid receptors are found in brain regions that contain dopaminergic neurons.[20–24] Activation of opioid receptors can modulate dopamine release, so that mu and delta opioid receptors cause an increase in dopamine release and activation of kappa opioid receptors produces a decrease in dopamine release.[25–28] In addition, behavioral data support a functional interaction between cocaine and the endogenous opioid system. In several different animal models of drug reinforcement, opioid receptor antagonists have been shown to attenuate the rewarding effects of cocaine.[29–35] These data support a role for central opioid systems in cocaine reinforcement. In addition, the mixed agonist-antagonist opioids nalbuphine and butorphanol have also been shown to reduce the self-administration of cocaine in monkeys;[36] however, this effect may be mediated by the agonist properties of the drugs. Cocaine also produces increased locomotor activity, which can be blocked by coadministration of opioid receptor antagonists.[30] Furthermore, behavioral sensitization to cocaine can be attenuated by opioid receptor antagonists.[37] Together these results suggest that the endogenous opioid system may be involved in mediating or modulating some of the effects of cocaine.

Studies have shown that different schedules of cocaine administration can produce different behavioral and neurochemical effects. For example, once-daily injections of cocaine are associated with increases in locomotor activity that become greater during repeated administration. This sensitization (defined as greater effect from a given dose of drug after previous exposure to the drug) is characteristic of repeated intermittent cocaine administration. If the same daily dose of cocaine is administered by continuous infusion, animals become tolerant (defined as a smaller effect from a given dose of drug after previous exposure to the drug) to the locomotor-stimulating effects of cocaine.[38–40] The same is true for the effects of cocaine on dopamine transporter function. Rats injected once daily with cocaine show enhanced inhibi-

tion of dopamine uptake by cocaine,[41] whereas rats receiving a constant infusion of cocaine by osmotic minipumps show attenuated inhibition of dopamine uptake by cocaine.[42] Furthermore, different degrees of sensitization are produced by different regimens of intermittent cocaine injections, with longer off-times producing greater sensitization.[38,43] Differences between the effects of intermittent versus steady-state administration of cocaine on neuroendocrine function have also been reported.[44,45] Therefore, the administration paradigm should be considered when evaluating the effects of cocaine on neurochemistry and behavior, and this may underlie some of the discrepancies found in the literature.

Current research on the regulation of the expression and function of opioid receptors as a consequence of exposure to cocaine is reviewed in this chapter.

REGULATION OF OPIOID RECEPTOR EXPRESSION AFTER COCAINE ADMINISTRATION

In vivo administration of cocaine can have profound effects on the expression of opioid receptors. We found that chronic repeated administration of cocaine can result in increased binding to mu and kappa opioid receptors.[46,47] In these studies, 60-day-old male Fischer rats were injected three times daily at 1-hour intervals for 14 days with cocaine, 10–45 mg/kg/day. This dosing paradigm was chosen to approximate the way that cocaine is often abused by humans (i.e., in a binge pattern), with frequent repeated self-administration over a short time interval followed by a period of abstinence.[48] Chronic administration of cocaine in this binge pattern produces behavioral sensitization.[49] Immediately after the last injection on day 14, brains were processed for quantitative receptor autoradiography under selective labeling conditions for mu, delta, and kappa opioid receptors. Results showed that binding to mu and kappa opioid receptors in specific brain regions was elevated in the cocaine-injected group as compared to the saline-injected control group. As shown in FIGURE 1, mu opioid receptors were upregulated in the rostral portion of the cingulate cortex, rostral nucleus accumbens, rostral caudate putamen, and basolateral amygdala after 14 days of cocaine administration.[46,47] FIGURE 2 shows the changes in binding to kappa and delta receptors after 14 days of binge-pattern cocaine administration. Binding to kappa opioid receptors was significantly increased in the rostral cingulate cortex, rostral caudate putamen, caudal olfactory tubercle, and ventral tegmental area.[47] In contrast to the upregulation of mu and kappa receptors, the density of delta opioid receptors was not significantly altered following 14 days of repeated cocaine administration in any brain region investigated.[47]

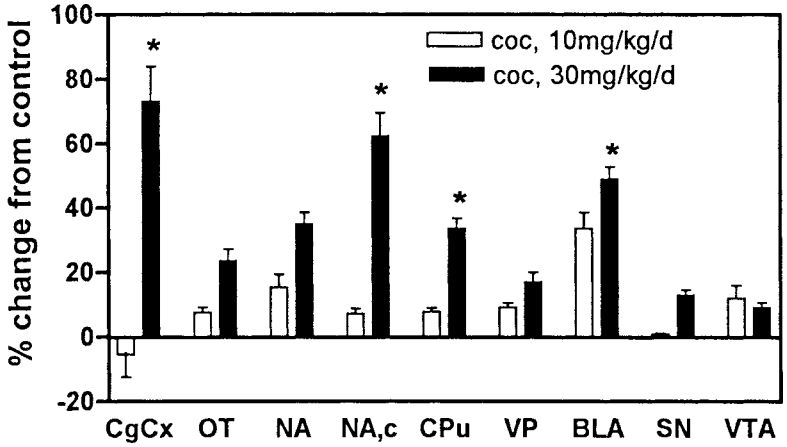

FIGURE 1. Effect of chronic binge-pattern cocaine administration on regional binding of [^{3}H]DAMGO to rat brain. Data shown as percent change in receptor density values in cocaine-treated animals as a function of receptor densities in control saline-injected animals. CgCx, rostral layer I of the cingulate cortex; OT, olfactory tubercle; NA, nucleus accumbens; NAc, rostral core of the nucleus accumbens; CPu, rostral caudate putamen; VP, ventral pallidum; BLA, basolateral amygdala; SN, substantia nigra; VTA, ventral tegmental area. *$p \leq 0.05$. Data taken from Unterwald *et al.*[46]

The time-course of mu receptor upregulation was examined by measuring mu opioid receptors after 1, 2, 7, or 14 days of binge-pattern cocaine administration. Mu receptor density in the cingulate cortex and caudate putamen increased gradually over time, becoming statistically significant by day 14 of cocaine administration. In contrast, significant upregulation of mu receptors in the nucleus accumbens was observed after 7 and 14 days of cocaine administration.[50] These results indicate that upregulation of mu opioid receptors occurs after chronic, but not acute, cocaine administration and that the nucleus accumbens appears to be more sensitive to the effects of cocaine than are other brain regions.

To investigate the mechanism involved in cocaine-induced mu opioid receptor changes, levels of messenger RNA that encodes the mu opioid receptor (MOR mRNA) were measured after binge-pattern cocaine administration using a solution hybridization RNase-protection assay. Male Fischer rats received three daily injections of cocaine, 15 mg/kg, or saline at 1-hour intervals in an identical manner to those just described. Results demonstrate a transient increase in MOR mRNA after the first day of cocaine administration. Thirty minutes following the third injection on day 1 of cocaine treatment, MOR mRNA levels were significantly elevated in areas of the

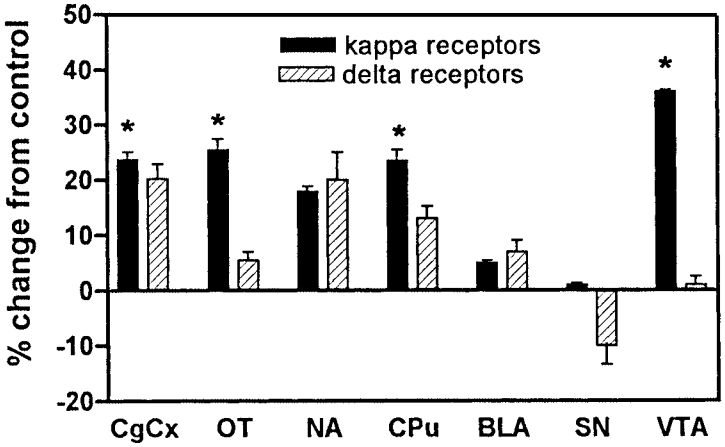

FIGURE 2. Effect of chronic binge-pattern cocaine administration, 45 mg/kg/day, on regional binding to kappa and delta opioid receptors in rat brain. Data shown as percent change in receptor density values in cocaine-treated animals as a function of receptor densities in control saline-injected animals. CgCx, rostral cingulate cortex; OT, caudal olfactory tubercle; NA, rostral nucleus accumbens; CPu, rostral caudate putamen; BLA, basolateral amygdala; SN, substantia nigra; VTA, ventral tegmental area. *$p \leq 0.05$. Data taken from Unterwald *et al.*[47]

mesolimbic and mesocortical dopamine system. MOR mRNA was increased in the frontal cortex (dissections included the rostral cingulate cortex), nucleus accumbens, and amygdala, but it was unchanged in the caudate putamen, hippocampus, thalamus, and hypothalamus.[51] The upregulation of MOR mRNA was transient, in that no significant changes in MOR mRNA were found after 7 or 14 days of repeated binge-pattern cocaine administration (Unterwald, Rubenfeld, and Kreek, unpublished data).

Of note is that brain regions that showed the greatest upregulation of mu and kappa opioid receptors after cocaine administration are areas where major dopaminergic pathways in the brain reside. Neurons of the nigrostriatal dopamine pathway terminate in the caudate putamen. Dopaminergic neurons of the mesocortical tract innervate the frontal cortex, cingulate cortex, and entorhinal cortex. The mesolimbic dopamine pathways project to the nucleus accumbens, olfactory tubercle, amygdaloid nuclei, and septum. In addition, dopamine transporter sites have a heterogeneous distribution throughout the striatum, with more rostral areas containing the highest density of binding sites.[52] The distribution of dopamine transporters matches the pattern of upregulation of opioid receptors, where the more rostral regions of the striatum showed the largest increases in receptor number. Because the brain areas where mu and kappa opioid receptors were upregulated are all enriched in

dopaminergic neurons and because cocaine inhibits dopamine reuptake, these results suggest that dopaminergic activity and alterations of such by cocaine may play a role in the regulation of mu and kappa opioid receptors.

Continuous chronic administration of cocaine has also been shown to alter opioid receptor densities. Hammer[53] and Hammer *et al.*[54] studied the effects of cocaine, 10 mg/kg/day, delivered by constant subcutaneous infusions via osmotic minipumps for 14 days to adult male Sprague-Dawley rats. Opioid receptors were measured by receptor autoradiography using [^{3}H]naloxone in a low, mu receptor-preferring concentration. [^{3}H]Naloxone binding was increased in the nucleus accumbens and in some of its projection sites such as the ventral pallidum and lateral hypothalamus after 14 days of continuous cocaine administration. Receptor densities were also increased in the medial geniculate and inferior colliculus, both of which are involved in auditory relay functions. Receptor densities in regions containing the cell bodies of ascending catecholamine systems, including the substantia nigra, ventral tegmental area, and dorsal raphe, showed reductions in receptor number, as did the basolateral amygdala after continuous cocaine exposure. No other brain regions were affected.[53] With the exception of the inferior colliculus and medial geniculate, changes in opioid receptor expression found in this study were confined to regions associated either directly or indirectly with central reward mechanisms. Opioid receptor levels were increased in the mesolimbic terminal and reward output regions, including the nucleus accumbens, ventral pallidum, and lateral hypothalamus, which are related directly to cocaine-induced reward.[13,16] The ventral tegmental area, substantia nigra, and dorsal raphe contain the cell bodies of dopaminergic and serotonergic neurons that project to the forebrain, and these regions exhibited decreases in opioid receptor number. Cells of the basolateral amygdaloid nucleus project to the accumbens, and hence reduction of opioid activity in this area could alter limbic output to the nucleus accumbens. Therefore, chronic continuous cocaine exposure decreased mu opioid receptor densities in regions that modulate neurochemical and functional projections to mesolimbic reward regions, while increasing receptor densities in the mesolimbic terminal regions.

Izenwasser and colleagues[55] have also shown upregulation of mu opioid receptors in the nucleus accumbens following chronic continuous administration of cocaine. In this study cocaine, 50 mg/kg/day, was administered to male Sprague-Dawley rats for 7 days by subcutaneously implanted osmotic minipumps. Binding of [^{3}H]DAMGO to mu opioid receptors in the nucleus accumbens and caudate putamen was measured 24 hours later, using homogenate binding assays. Mu receptor density in the nucleus accumbens was about 23% higher in animals receiving cocaine infusions than in control animals. No significant changes were found in the caudate putamen.[55]

In similarly treated animals, MOR mRNA levels were transiently elevated following chronic continuous cocaine administration as measured by quantitative RT-PCR.[56,57] MOR mRNA was significantly increased in the nucleus accumbens, but not in the caudate putamen or olfactory bulb, following 2 or 3 days of continuous cocaine administration in male Sprague-Dawley rats.[56,57] MOR mRNA returned to baseline levels by day 4 of continuous cocaine administration. Once again, this effect was selective for mu opioid receptors in that no changes were noted in mRNA levels for delta opioid receptors after 3 days of continuous cocaine administration. To determine if dopamine receptors play a role in the regulation of MOR mRNA by cocaine, selective dopamine receptor antagonists and agonists were investigated. Cocaine-induced MOR mRNA upregulation was blocked by coadministration of either D1, D2, or D3 dopamine receptor antagonists.[56,58] Furthermore, elevations in MOR mRNA were also found after continuous administration of other direct and indirect dopamine agonists.[58] These data confirm the involvement of dopaminergic mechanisms in the effects of cocaine on mu opioid receptor regulation.

Gestational exposure to cocaine also increases opioid receptor binding in weanling rat offspring.[54,59] In this study, cocaine, 10–40 mg/kg, was administered to pregnant dams once daily from embryonic day 8 (E8) to E20 by subcutaneous injections. Receptor densities were measured in male offspring on postnatal day 21 by *in vitro* receptor autoradiography using [^{3}H]naloxone under conditions of preferential mu receptor binding. Prenatal cocaine exposure resulted in a dose-dependent increase in opioid receptor number in dopaminergic terminal regions including the nucleus accumbens, medial prefrontal cortex, olfactory tubercle, and caudate putamen; in limbic areas including the basolateral amygdala, lateral habenula, hippocampus, entorhinal cortex, and cingulate cortex; and in neocortical regions including somatosensory and motor cortices.[59] The upregulation of mu receptors produced by gestational cocaine exposure in brain regions containing dopaminergic terminals is consistent with a direct dopamine-opioid interaction at these sites. However, because many of the regions affected by cocaine in this study are thought not to be involved in cocaine reinforcement or to contain dopaminergic terminals, gestational cocaine exposure seems to produce more extensive, less specific effects on developing opioid systems than those produced in adult animals. These data also demonstrate that gestational cocaine exposure can produce long-term effects on opioid receptor binding, which were assessed 24 days after cessation of cocaine administration in this study.

Interestingly, Rhesus macaque fetuses that were exposed to cocaine from E22 to E70 showed reduced levels of MOR mRNA in the diencephalon at gestational day 70.[60] The differences in findings between these two studies could be due to species-specific effects or to the age at which receptors were

examined. Also, mRNA levels and protein levels are not always regulated in a coordinated manner.

Together, results from these animal studies [46,47,53,55,59] suggest that chronic exposure to cocaine can result in alterations in opioid receptor expression. Although some discrepancies in the findings may be due to procedural differences in drug administration or receptor measurements or to animals strain differences, upregulation of mu opioid receptors in the nucleus accumbens is a common finding among most, if not all, of these studies. Therefore, mu opioid receptor activity in this brain region may be critical for cocaine reinforcement.

Compelling evidence supports the importance of mu opioid receptors in human cocaine addiction. The finding of increased mu receptor binding following chronic cocaine administration in rats has been substantiated and extended in human cocaine-dependent men.[61] Mu opioid receptor binding was measured in 10 cocaine-dependent men and 7 nonaddicted control subjects using positron emission tomography (PET) with [^{11}C]carfentanil. Results of this experiment demonstrate that mu receptor binding was increased in several brain regions of the cocaine addicts when studied 1 to 4 days after their last use of cocaine. Mu receptor binding was significantly increased in the caudate nucleus, thalamus, cingulate cortex, frontal cortex, and temporal cortex. At the time of the PET scan, the severity of craving for cocaine was also measured using the Minnesota Cocaine Craving Scale and visual analog scales. Self-reported craving was positively correlated with mu receptor binding in the amygdala, anterior cingulate cortex, frontal cortex, and temporal cortex. After an additional 4 weeks of monitored abstinence, mu opioid receptor binding remained increased in most brain regions, although there was no longer a significant correlation with cocaine craving. The investigators hypothesized that the upregulation of mu receptors may be due to a chronic reduction in endogenous opioid peptide release, which could also mediate some of the dysphoria experienced by cocaine addicts between episodes of cocaine use.[61] These findings demonstrate the involvement of mu opioid receptors in cocaine dependence and craving in living human subjects.

Studies on postmortem human brain samples from persons who died from cocaine overdose have revealed marked increases in kappa opioid receptors. As shown in FIGURE 3, kappa receptor binding was significantly increased in the nucleus accumbens and other corticolimbic areas of the cocaine overdose victims as compared to samples from age-matched drug-free control subjects.[62,63] In cocaine overdose victims who experienced paranoia and marked agitation before death, kappa receptor density was also elevated in the amygdala.[62,63] Another study reported an increase in kappa opioid receptor binding in the caudate nucleus of subjects with a history of cocaine abuse and a positive cocaine toxicology at the time of death.[64] Interestingly, despite the

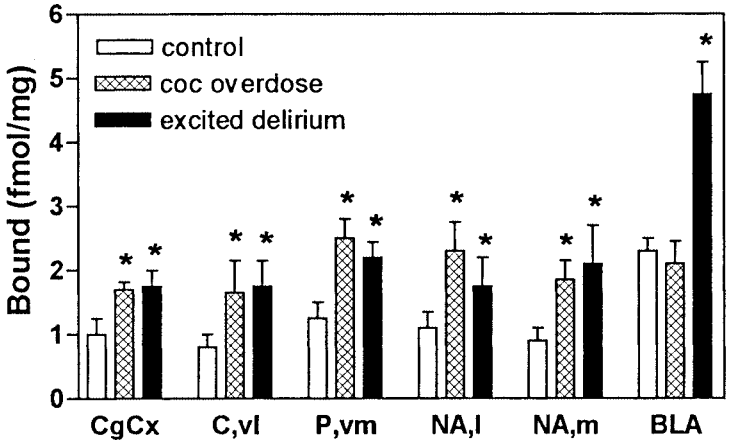

FIGURE 3. [^{125}I]IOXY binding to kappa receptors in brain regions of interest from drug-free control subjects, cocaine overdose victims, and cocaine abusers who had excited delirium at the time of death. CgCx, anterior cingulate cortex; Cvl, anterior ventrolateral caudate; Pvm, anterior ventromedial putamen; NAl, lateral nucleus accumbens; NAm, medial nucleus accumbens; BLA, basolateral amygdala. *p <0.05. Data taken from Staley *et al.*[62]

many reports of cocaine-induced upregulation of kappa opioid receptor binding in both animals and humans, kappa receptor mRNA levels were reported to be decreased after cocaine administration in rat substantia nigra,[65] nucleus accumbens,[66] and ventral tegmental area.[66]

The mechanisms by which cocaine produces alterations in opioid receptor expression are unknown, but experimental evidence suggests that this effect of cocaine is mediated, at least in part, by dopamine. Continuous administration of the selective dopamine reuptake inhibitor RTI-117 for 14 days resulted in upregulation of mu opioid receptors in the caudate putamen and nucleus accumbens (Izenwasser, unpublished results). Striatal mu opioid receptors were increased following 6 days of continuous infusion of the D2 dopamine receptor agonist quinpirole, but not after infusion of the D1 receptor agonist SKF 38393.[67] This effect was selective for mu receptors, in that binding to delta opioid receptors was unchanged after either quinpirole or SKF 38393. The authors of this study suggest that the upregulation of mu receptor by quinpirole may be a compensatory response to dopaminergic inhibition of enkephalin biosynthesis and, indeed, proenkephalin mRNA levels were decreased after this treatment.[67] In agreement, chronic administration of D2 dopamine receptor antagonists has been shown to produce a decrease in mu opioid receptor binding in rat striatum.[68,69] Together, these data lend further support to the idea that chronic cocaine administration produces an upregu-

lation in mu opioid receptors due to inhibition of dopamine reuptake and activation of D2 dopamine receptors.

REGULATION OF OPIOID RECEPTOR-MEDIATED SIGNAL TRANSDUCTION AFTER COCAINE ADMINISTRATION

As summarized in the foregoing section, chronic administration of cocaine using a variety of paradigms can produce alterations in opioid receptor expression. The functional consequences of changes in opioid receptor expression have been studied to a limited extent. Opioid receptors are coupled to inhibitory G-proteins, Gi and Go, which regulate enzyme activity and ion channel functioning. One enzyme whose activity is regulated by opioid receptors via G-proteins is adenylyl cyclase, which converts ATP into cAMP. Activation of opioid receptors inhibits the activity of adenylyl cyclase and thus reduces cAMP formation.[70,71]

Adenylyl cyclase activity has been used as a functional measure of opioid receptor-mediated signal transduction. We investigated the functional consequences of cocaine-induced opioid receptor upregulation by measuring adenylyl cyclase activity in the nucleus accumbens and caudate putamen of cocaine-treated rats. As in receptor expression studies, male Fischer rats re-

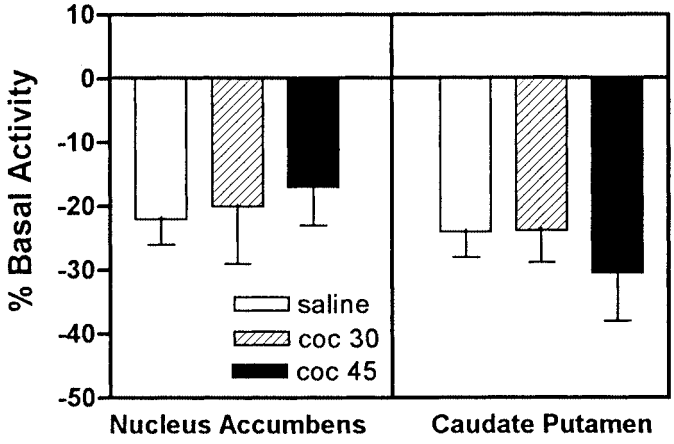

FIGURE 4. Inhibition of basal adenylyl cyclase activity in the nucleus accumbens and caudate putamen by the selective mu receptor agonist DAMGO (10^{-5} M) in animals injected with saline or cocaine, 30 or 45 mg/kg/day, in a binge pattern for 14 days. Data taken from Unterwald *et al.*[72]

ceived three daily injections of cocaine in a binge pattern for 14 days and were euthanized 30 minutes after the last injection. As just described, this cocaine treatment resulted in upregulation of mu opioid receptors in the nucleus accumbens and caudate putamen and no alterations in delta receptor number.[46,47] Adenylyl cyclase activity was assessed by determining the amount of cAMP formed in tissue homogenates from the nucleus accumbens and caudate putamen in the presence of mu and delta opioid receptor agonists.[72]

Results demonstrate that the selective mu opioid receptor agonist DAMGO inhibited adenylyl cyclase activity in a concentration-dependent manner in saline-injected control animals, with a maximal inhibition of 24% in the caudate putamen and 22% in the nucleus accumbens. Chronic repeated cocaine administration did not alter the inhibition of adenylyl cyclase activity by DAMGO in either brain region. FIGURE 4 illustrates the inhibition of basal adenylyl cyclase activity by DAMGO in the nucleus accumbens and caudate putamen of saline- and cocaine-injected rats. The selective delta opioid receptor agonist DPDPE produced maximal inhibition of adenylyl cyclase activity of 31% in both the caudate putamen and the nucleus accumbens. In animals that received chronic repeated cocaine administration, maximal inhibition of cyclase activity by DPDPE was reduced to only 2% in animals receiving 30 mg/kg/day and 11% in those receiving 45 mg/kg/day in the caudate putamen. Similar results were found in the nucleus accumbens. Maximal inhibition produced by DPDPE was 3% in animals receiving 30 mg/kg/day and 13% in those receiving 45 mg/kg/day in the accumbens,[72] as shown in FIGURE 5.

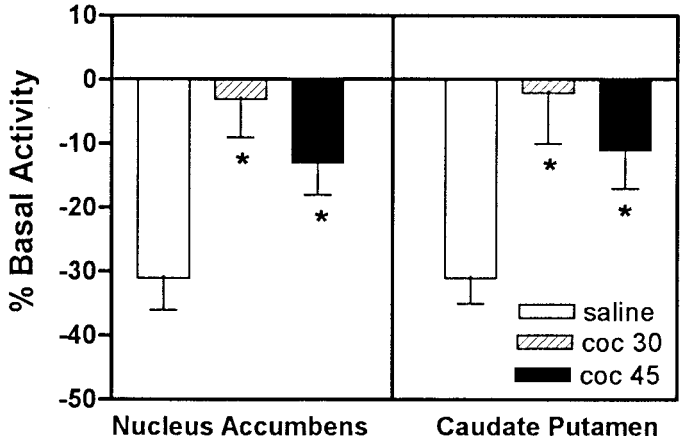

FIGURE 5. Inhibition of basal adenylyl cyclase activity in the nucleus accumbens and caudate putamen by the selective delta receptor agonist DPDPE (10^{-5} M) in animals injected with saline or cocaine, 30 or 45 mg/kg/day, in a binge pattern for 14 days. *$p \leq 0.05$. Data taken from Unterwald *et al.*[72]

Attenuation of delta receptor-regulated cyclase activity was unexpected, because no change in delta receptor binding was found in animals treated with cocaine by the same regimen.[46,47] The data suggest that chronic repeated cocaine administration results in selective attenuation of delta opioid receptor-mediated signal transduction through the cAMP pathway in both the nucleus accumbens and the caudate putamen. Because the number of delta receptors does not appear to be altered after cocaine administration, it is possible that exposure to cocaine results in an uncoupling of delta receptors from G-proteins. Results from this study failed to detect significant changes in mu receptor-inhibition of adenylyl cyclase activity despite the finding of an upregulation of mu opioid receptors in both of these brain regions after cocaine administration. Because cocaine administration produced a 30–40% increase in mu receptor number, it is possible that the corresponding changes in adenylyl cyclase activity were too small to detect by this method. This is based on the finding from another study that chronic treatment with naltrexone produced a 2.3-fold increase in mu receptor expression, but only a 40% increase in the efficacy of DAMGO to inhibit adenylyl cyclase activity.[73]

We further explored the mechanisms involved in the attenuation of delta receptor signal transduction following chronic repeated cocaine administration.[74] Results from these studies demonstrated that chronic repeated administration of the selective D1 dopamine receptor agonist SKF 82958, but not the D2 dopamine receptor agonist quinpirole, also attenuates the ability of delta opioid receptors to inhibit adenylyl cyclase activity. Similar to the findings with cocaine, binding to delta opioid receptors was unchanged after administration of SKF 82958 or quinpirole. These results suggest that chronic repeated activation of D1 dopamine receptors, either with a D1 receptor agonist or indirectly with cocaine, is involved in the loss of delta opioid receptor signaling. The coupling of delta receptors to G-proteins was investigated by measuring the binding of [^{35}S]GTPgS to G-proteins after activation of delta receptors by DPDPE.[75] Results demonstrated that repeated administration of SKF 82958 resulted in a decrease in the ability of DPDPE to stimulate [^{35}S]GTPgS binding in both the nucleus accumbens and the caudate putamen.[74]

Together, these data demonstrate that activation of dopaminergic systems can produce significant modulation of opioid receptor function. Chronic repeated activation of D1 dopamine receptors by administration of SKF 82958 or cocaine attenuates delta opioid receptor inhibition of adenylyl cyclase activity in the nucleus accumbens and caudate putamen. This finding suggests that cocaine-induced desensitization of delta opioid receptor function[72] is mediated by D1 dopamine receptors.[74] Furthermore, this desensitization is due, at least in part, to reduced coupling between delta receptors and inhibitory G-proteins. The reduced inhibition of adenylyl cyclase activity by delta

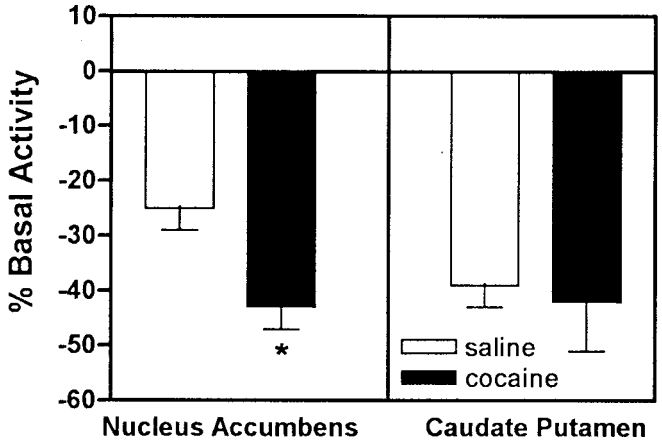

FIGURE 6. Inhibition of basal adenylyl cyclase activity in the nucleus accumbens and caudate putamen by the selective mu receptor agonist DAMGO (10^{-5} M) in animals treated with saline or cocaine, 50 mg/kg/day, by continuous subcutaneous infusion for 7 days. Data taken from Izenwasser *et al.*[55]

receptors may result in elevated levels of cAMP. Because cAMP mediates many intracellular events that are involved in long-term cellular plasticity, including regulation of gene transcription, some of the behavioral and physiological sequelae seen after administration of psychostimulants may be mediated by changes in the adenylyl cyclase signal transduction system.

The effects of chronic continuous cocaine administration on adenylyl cyclase activity were also investigated.[55] Male Sprague-Dawley rats were treated with cocaine, 50 mg/kg/day, or saline for 7 days by subcutaneously implanted osmotic minipumps. As just described, this treatment resulted in upregulation of mu opioid receptors in the nucleus accumbens.[55] Chronic continuous cocaine exposure increased the maximal inhibition of adenylyl cyclase activity by the mu receptor agonist DAMGO in the nucleus accumbens from 25% to 43%, as shown in FIGURE 6. No significant alterations in delta receptor-inhibited cyclase activity were found (FIG. 7). Also, there were no changes in mu (FIG. 6) or delta (FIG. 7) opioid receptor-regulated cyclase activity in the caudate putamen. These results further illustrate that the administration paradigm can influence the effects of cocaine exposure. Intermittent cocaine administration produced a loss of delta opioid receptor-mediated adenylyl cyclase inhibition in the nucleus accumbens and caudate putamen,[72] whereas continuous cocaine exposure selectively increased mu receptor-mediated cyclase inhibition in the nucleus accumbens only.[55]

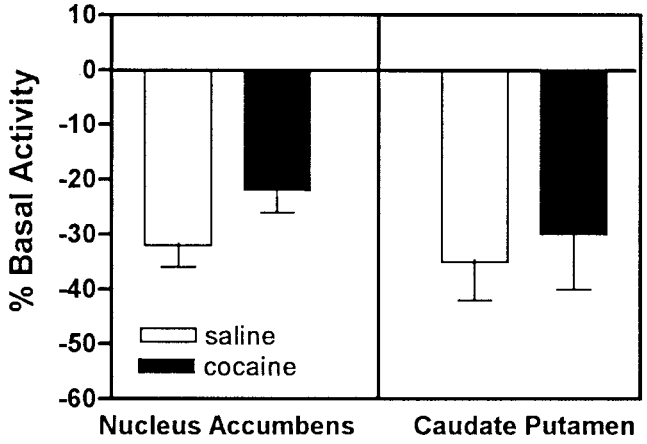

FIGURE 7. Inhibition of basal adenylyl cyclase activity in the nucleus accumbens and caudate putamen by the selective delta receptor agonist DPDPE (10^{-5} M) in animals treated with saline or cocaine, 50 mg/kg/day, by continuous subcutaneous infusion for 7 days. Data taken from Izenwasser *et al.*[55]

SUMMARY AND CONCLUSIONS

Data from the current literature support the idea that cocaine has profound effects on the expression and function of central opioid receptors. Cocaine administered to a variety of species using many different treatment regimens results in alterations in the expression of opioid receptors. A detailed consensus about the exact nature of the changes is lacking, probably because of the different schedules of cocaine administration and the different species and strains of animals used in these studies. A consistent finding, however, is the upregulation of mu opioid receptors in the nucleus accumbens and regions of the mesocorticolimbic dopamine system. Another consistent finding is the lack of regulation of delta opioid receptor expression. The mechanism underlying the regulation of mu and kappa opioid receptors by cocaine has not been completely elucidated, but dopamine working through activation of D2 dopamine receptors may be involved.

Opioid receptor function has also been shown to be affected by chronic cocaine exposure. Inhibition of adenylyl cyclase activity by opioid receptors is altered after cocaine exposure. Again, the schedule of cocaine administration appears to influence cocaine's effect on cyclase activity. Intermittent cocaine administration produces a loss of delta receptor-mediated inhibition of adenylyl cyclase activity in the nucleus accumbens and caudate putamen. The at-

tenuation of delta opioid receptor-mediated signal transduction after cocaine administration appears to be mediated by activation of D1 dopamine receptors and is due, at least in part, to an uncoupling of delta receptors from inhibitory G-proteins. In contrast, continuous administration of cocaine selectively increases mu opioid receptor-inhibited adenylyl cyclase activity in the nucleus accumbens.

The regulation of opioid receptors and also endogenous opioid peptides during exposure to cocaine is clearly evident at cellular and molecular levels. The functional significance of this regulation is not completely understood, but behavioral studies indicate that opioid systems play a role in cocaine-induced behaviors. Studies in human cocaine addicts also support the importance of the endogenous opioid system in cocaine abuse and cocaine craving. Therefore, cocaine-induced changes in opioid receptor expression and function may contribute to the perpetuation of cocaine abuse and/or to relapse.

ACKNOWLEDGMENTS

Much of the work described in this chapter was supported by NIDA DA09580 (E.M.U.) and P50-DA05130 (M.J. Kreek).

REFERENCES

1. HEIKKILA, R.E., H. ORLANSKY & G. COHEN. 1975. Studies on the distinction between uptake inhibition and release of [^{3}H]dopamine in rat brain tissue slices. Biochem. Pharmacol. **24:** 847–852.
2. RITZ, M.C., R.J. LAMB, S.R. GOLDBERG, *et al*. 1987 Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science **237:** 1219–1223.
3. KILTY, J.E., D. LORANG & S.G. AMARA. 1991. Cloning and expression of a cocaine-sensitive rat dopamine transporter. Science **254:** 578–579.
4. SHIMADA, S., S. KITAYAMA, C.L. LIN, *et al*. 1991. Cloning and expression of a cocaine-sensitive dopamine transporter complementary DNA. Science **254:** 576–578.
5. GAINETDINOV, R.R., W.C. WETSEL, S.R. JONES, *et al*. 1999. Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science. **283:** 397–401.
6. ROCHA, B.A., F. FUMAGALLI, R.R. GAINETDINOV, *et al*. 1998. Cocaine self-administration in dopamine-transporter knockout mice. Nat. Neurosci. **1:** 132–137.
7. ROCHA, B.A., K. SCEARCE-LEVIE, J.J. LUCAS, *et al*. 1998. Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. Nature **393:** 175–178.
8. WIECZOREK, W.J. & Z.L. KRUK. 1994. A quantitative comparison on the effects of beztyropine, cocaine and nomifensine on electrically evoked dopamine

overflow and rate of re-uptake in the caudate putamen and nucleus accumbens in the rat brain slice. Brain Res. **657:** 42–50.
9. ROSS, S.B. & A.L. RENYI. 1969. Inhibition of the uptake of tritiated 5-hydroxytryptamine in brain tissue. Eur. J. Pharmacol. **7:** 270–277.
10. HADFIELD, M.G., D.E.W. MOTT & J.A. ISMAY. 1980. Cocaine: Effect of *in vivo* administration on synaptosomal uptake of norepinephrine. Biochem. Pharmacol. **29:** 1861–1863.
11. FLEMING, J.A., R. BYCK & P.J. BARASH. 1990. Pharmacology and therapeutic applications of cocaine. Anesthesiology **73:** 518–531.
12. WISE, R.A. & M.A. BOZARTH. 1984. Brain reward circuitry: four circuit elements wired in apparent series. Brain Res. Bull. **12:** 203–208.
13. KOOB, G.F., F.J. VACCARINO, M. AMALRIC, *et al.* 1987. Neural substrates for cocaine and opiate reinforcement. *In* Cocaine: Clinical and Behavioral Aspects. S. Fisher, A. Raskin & E.H. Uhlenhuth, eds. :80–108. Oxford Press. New York, NY.
14. ROBERTS, D.C.S., G.F. KOOB, P. KLONOFF, *et al*. 1980. Extinction and recovery of cocaine self-administration following 6-hydroxydop[amine lesions of the nucleus accumbens. Pharmacol. Biochem. Behav. **12:** 781–787.
15. GOEDERS, N.E. & J.E. SMITH. 1983. Cortical dopaminergic involvement in cocaine reinforcement. Science **221:** 773–775.
16. HUBNER, C.B. & G.F. KOOB. 1990. The ventral pallidum plays a role in mediating cocaine and heroin self-administration in the rat. Brain Res. **508:** 20–29.
17. KORNETSKY C., D. HUSTON-LYONS & L.J. PORRINO. 1991. The role of the olfactory tubercle in the effects of cocaine, morphine and brain-stimulation reward. Brain Res. **541:** 75–81.
18. FINK, J.S. & G.P. SMITH. 1980. Mesolimbocortical dopamine terminal fields are necessary for normal locomotor and investigatory exploration in rats. Brain Res. **199:** 359–385.
19. ROBERTS, D.C.S. & G.F. KOOB. 1982. Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. Pharmacol. Biochem. Behav. **17:** 901–904.
20. KHACHATURIAN, H., M.E. LEWIS, V. HOLLT, *et al.* 1983. Telencephalic enkephalinergic systems in the rat brain. J. Neurosci. **3:** 844–855.
21. FALLON, J.J. & F.M. LESLIE. 1986. Distribution of dynorphin and enkephalin peptides in the rat brain. J. Comp. Neurol. **249:** 293–336.
22. MANSOUR, A., M.E. LEWIS, H. KHACHATURIAN, *et al*. 1986. Pharmacological and anatomical evidence of selective mu, delta, and kappa opioid receptor binding in rat brain. Brain Res. **399:** 69–79
23. TEMPEL, A. & R.S. ZUKIN. 1987. Neuroanatomical patterns of the mu, delta, and kappa opioid receptors of rat brain as determined by quantitative *in vitro* autoradiography. Proc. Natl. Acad. Sci. USA **84:** 4308–4312.
24. MANSOUR, A., C.A. FOX, H. AKIL, *et al.* 1995. Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. TINS **18:** 22–29.
25. BIGGIO, G., M. CASU, M.G. CORDA, *et al*. 1978. Stimulation of dopamine synthesis in caudate nucleus by intrastriatal enkephalins and antagonism by naloxone. Science **200:** 552–554.
26. WOOD, P.L., M. STATLAND, J.W. RICHARD, *et al.* 1980. Actions of mu, kappa, sigma, delta and agonist/antagonist opiates on striatal dopaminergic function. J. Pharmacol. Exp. Ther. **215:** 697–703.

27. SPANAGEL, R., A. HERZ & T.S. SHIPPENBERG. 1990. The effects of opioid peptides on dopamine release in the nucleus accumbens: an *in vivo* microdialysis study. J. Neurochem. **55:** 1734–1740.
28. SPANAGEL, R., A. HERZ & T.S. SHIPPENBERG. 1992. Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proc. Natl. Acad. Sci. USA **89:** 2046–2050.
29. BAIN, G.T. & C. KORNETSKY. 1987. Naloxone attenuation of the effect of cocaine on rewarding brain stimulation. Life Sci. **40:** 1119–1125.
30. HOUDI, A.A., M.T. BARDO & G.R. VAN LOON. 1989. Opioid mediation of cocaine-induced hyperactivity and reinforcement. Brain Res. **497:** 195–198.
31. BILSKY, E.J., M.J. MONTEGUT, C.L. DELON, *et al*. 1992. Opioidergic modulation of cocaine conditioned place preferences. Life Sci. **50:** PL85–90.
32. CORRIGAL, W.A. & K.M. COEN. 1991. Opiate antagonists reduce cocaine but not nicotine self-administration. Psychopharmacology **104:** 167–170.
33. MELLO, N.K., J.H. MENDELSON, M. BREE, *et al*. 1990. Buprenorphine and naltrexone effects on cocaine self-administration by rhesus monkeys. J. Pharmacol. Exp. Ther. **254:** 926–939.
34. REID, L.D., S.D. GLICK, K.A. MENKENS, *et al.* 1995. Cocaine self-administration and naltrindole, a delta-selective opioid antagonist. Neuroreport **6:** 1409–1412.
35. KUZMIN, A.V., M.A. GERRITS & J.M. VAN REE. 1998. Kappa-opioid receptor blockade with nor-binaltorphimine modulates cocaine self-administration in drug-naive rats. Eur. J. Pharmacol. **358:** 197–202.
36. MELLO, N.K., J.B. KAMIEN, S.E. LUKAS, *et al.* 1993. The effects of nalbuphine and butorphanol treatment on cocaine and food self-administration by rhesus monkeys. Neuropsychopharmacology **8:** 45–49.
37. HEIDBREDER, C.A., S.R. GOLDBERG & T.S. SHIPPENBERG. 1993. Inhibition of cocaine-induced sensitization by the delta-opioid receptor antagonist naltrindole. Eur. J. Pharmacol. **243:** 123–127.
38. POST, R.M. 1980. Intermittent versus continuous stimulation: effect of time interval on the development of sensitization or tolerance. Life Sci. **26:** 1275–1282.
39. REITH, M.E.A., M. BENUCK & A. LAJTHA. 1987. Cocaine disposition in the brain after continuous or intermittent treatment and locomotor stimulation in mice. J. Pharmacol. Exp. Ther. **243:** 281–287.
40. ZEIGLER, S., J. LIPTON, A. TOGA, *et al.* 1991. Continuous cocaine administration produces persisting changes in brain neurochemistry and behavior. Brain Res. **552:** 27–35.
41. IZENWASSER, S. & B.M. COX. 1990. Daily cocaine treatment produces a persistent reduction of [^{3}H]dopamine uptake *in vitro* in rat nucleus accumbens but not in striatum. Brain Res. **531:** 338–341.
42. IZENWASSER, S. & B.M. COX. 1992. Inhibition of dopamine uptake by cocaine and nicotine: tolerance to chronic treatment. Brain Res. **573:** 119–125.
43. KALIVAS, P.W. & J. STEWART. 1991. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. **16:** 223–244.
44. KREEK, M.J. 1983. Factors modifying the pharmacological effectiveness of methadone. *In* Research in the Treatment of Narcotic Addictions: State of the Art. J.R. Cooper, F. Altman, B.S. Brown & D. Czechowicz, eds. :444–450. NIDA Res. Monograph. Bethesda. MD.

45. TORRES, G. & C. RIVIER. 1992. Differential effects of intermittent or continuous exposure to cocaine on the hypothalamic-pituitary-adrenal axis and c-fos expression. Brain Res. **571:** 204–211.
46. UNTERWALD, E.M., J. HORNE-KING & M.J. KREEK. 1992. Chronic cocaine alters brain mu opioid receptors. Brain Res. **584:** 314–318.
47. UNTERWALD, E.M., J.M. RUBENFELD & M.J. KREEK. 1994. Repeated cocaine administration upregulates kappa and mu, but not delta opioid receptors. NeuroReport **5:** 1613–1616.
48. GAWIN, F.H. 1991. Cocaine addiction: psychology and neurophysiology. Science **251:** 1580–1586
49. UNTERWALD, E.M., A. HO, J.M. RUBENFELD, *et al*. 1994. Time course of the development of behavioral sensitization and dopamine receptor upregulation during binge cocaine administration. J. Pharmacol. Exp. Ther. **270:** 1387–1397.
50. UNTERWALD, E.M., J.M. RUBENFELD & M.J. KREEK. 1995. Time course of cocaine-induced alterations in opioid and dopamine receptors and transporter sites. NIDA Research Monograph **153:** 507.
51. YUFEROV, V., Y. ZHOU, R. SPANGLER, *et al*. 1999. Acute "binge" cocaine increases mu-opioid receptor mRNA levels in areas of the rat mesolimbic mesocortical dopamine system. Brain Res. Bull. **48:** 109–112.
52. GRAYBIEL, A.M. & R. MORATALLA. 1989. Dopamine uptake sites in the striatum are distributed differentially in striosome and matrix compartments. Proc. Natl. Acad. Sci. USA **86:** 9020–9024 .
53. HAMMER, R.P., JR. 1989. Cocaine alters opiate receptor binding in critical brain reward regions. Synapse **3:** 55–60.
54. HAMMER, R.P., JR., D.W. CLOW & L.P. SPEAR. 1991. Opioid involvement in the rewarding effects of chronic cocaine. NIDA Research Monograph **105:** 182–188.
55. IZENWASSER, S., B. HELLER & B.M. COX. 1996. Continuous cocaine administration enhances mu- but not delta-opioid receptor-mediated inhibition of adenylyl cyclase activity in nucleus accumbens. Eur. J. Pharmacol. **297:** 187–191.
56. AZARYAN, A.V., L.J. COUGHLIN, B. BUZAS, *et al.* 1996. Effect of chronic cocaine treatment on mu- and delta-opioid receptor mRNA levels in dopaminergically innervated brain regions. J. Neurochem. **66:** 443–448.
57. AZARYAN, A.V., B.J. CLOCK, J.G. ROSENBERGER, *et al.* 1998. Transient upregulation of mu opioid receptor mRNA levels in nucleus accumbens during chronic cocaine administration. Can. J. Physiol. Pharmacol. **76:** 278–283.
58. AZARYAN, A.V., B.J. CLOCK & B.M. COX. 1996. Mu opioid receptor mRNA in nucleus accumbens is elevated following dopamine receptor activation. Neurochem. Res. **21:** 1411–1415.
59. CLOW, D.W., R.P. HAMMER, JR., C.L. KIRSTEIN, *et al.* 1991. Gestational cocaine exposure increases opiate receptor binding in weanling offspring. Dev. Brain. Res. **59:** 179–185.
60. CHAI, L., M.A. BOSCH, J.M. MOORE, *et al.* 1999. Chronic prenatal cocaine treatment down-regulates mu-opioid receptor mRNA expression in the brain of fetal Rhesus Macaque. Neurosci. Lett. **261:** 45–48.
61. ZUBIETA, J.-K., D.A. GORELICK, R. STAUFFER, *et al.* 1996. Increased mu opioid receptor binding detected by PET in cocaine-dependent men is associated with cocaine craving. Nature Med. **2:** 1225–1229.

62. STALEY, J.K., R.B. ROTHMAN, K.C. RICE *et al.* 1997. Kappa2 opioid receptors in limbic areas of the human brain are upregulated by cocaine in fatal overdose victims. J. Neurosci. **17:** 8225–8233.
63. MASH, D.C. & J.K STALEY. 1999. D3 dopamine and kappa opioid receptor alterations in human brain of cocaine-overdose victims. Ann. NY Acad. Sci. **877:** 507–522.
64. HURD, Y.L. & M. HERKENHAM. 1993. Molecular alterations in the neostriatum of human cocaine addicts. Synapse **13:** 357–369.
65. SPANGLER, R., A. HO, Y. ZHOU, *et al*. 1996. Regulation of kappa opioid receptor mRNA in the rat brain by 'binge' pattern cocaine administration and correlation with preprodynorphin mRNA. Mol. Brain Res. **38:** 71–76.
66. ROSIN, A., S. LINDHOLM, J. FRANCK, *et al.* 1999. Downregulation of kappa opioid receptor mRNA levels by chronic ethanol and repetitive cocaine in rat ventral tegmentum and nucleus accumbens. Neurosci. Lett. **275:** 1–4.
67. CHEN, J.F., V.J. ALOYO & B. WEISS. 1993. Continuous treatment with the D2 dopamine receptor agonist quinpirole decreases D2 dopamine receptors, D2 dopamine receptor messenger RNA and proenkephalin messenger RNA, and increases mu opioid receptors in mouse striatum. Neuroscience **54:** 669–680.
68. TROVERO, F., D. HERVE, M. DESBAN, *et al.* 1990. Striatal opiate mu receptors are not located on dopamine nerve endings in the rat. Neuroscience **39:** 313–321.
69. CHEN, J.F., V.J. ALOYO, Z.-H. QIN, *et al*. 1994. Irreversible blockade of D2 dopamine receptors by fluphenazine-N-mustard increases D2 dopamine receptor mRNA and proenkephalin mRNA and decreases D1 receptor mRNA and mu and delta opioid receptors in rat striatum. Neurochem. Int. **25:** 355–366.
70. KLEE, W.A., S.K. SHARMA & M. NIRENBERG. 1975. Opiate receptors as regulators of adenylate cyclase. Life Sci. **16:** 1869–1874.
71. CHILDERS, S.R. 1988. Opiate-inhibited adenyate cyclase in rat brain membranes depleted of GS-stimulated adenyate cyclase. J. Neurochem. **50:** 543–553.
72. UNTERWALD, E.M., B.M. COX, M.J. KREEK, *et al*. 1993. Chronic repeated cocaine administration alters basal and opioid-regulated adenylyl cyclase activity. Synapse **15:** 33–38.
73. COTE, T.E., S. IZENWASSER & H.B. WEEMS. 1993. Naltrexone-induced upregulation of mu-opioid receptors 7315c cell and brain membranes: enhancement of opioid efficacy in inhibiting adenylyl cyclase. J. Pharmacol. Exp. Ther. **267:** 238–244.
74. UNTERWALD, E.M. & M. CUNTAPAY. 2000. Dopamine-opioid interactions in the rat striatum: a modulatory role for dopoamine D1 receptors in delta opioid receptor-mediated signal transduction. Neuropharmacology **39:** 372–381.
75. SIM, L.J., D.E. SELLEY & S.R. CHILDERS. 1995. *In vitro* autoradiography of receptor-activated G proteins in rat brain by agonist-stimulated guanylyl 5′-[g-[35S]thio]-triphosphate binding. Proc. Natl. Acad. Sci. USA **92:** 7242–7246.