

In Vivo Regulation of μ -Opioid Receptor Density and Gene Expression in CXBK and Outbred Swiss Webster Mice

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KEY WORDS μ -opioid receptor; mouse μ -opioid receptor mRNA; receptor density and affinity; downregulation; tolerance; etorphine; DAMGO; CXBK; Swiss Webster

ABSTRACT Chronic in vivo treatment with the opioid agonist etorphine downregulates μ -opioid receptor density, produces tolerance, and regulates gene expression in the mouse. After cessation of treatment, there is an increase in μ -opioid receptor mRNA level associated with the recovery of μ -opioid receptors. However, the effect of etorphine on the regulation of mRNA during treatment is currently not known. In this study, etorphine-induced changes in μ -opioid receptor density, mRNA, and opioid analgesic potency were determined in two mouse strains that differ in basal μ -opioid receptor density in brain. CXBK mice (μ -opioid receptor deficient) and outbred Swiss Webster mice were implanted s.c. with placebo pellets (controls) or etorphine minipumps (250 μ g/kg/day) for 1–7 days and μ -opioid receptor density or mRNA levels in whole brain were assessed or mice were tested for etorphine analgesia following 7 days of treatment. In control CXBK mice, μ -receptor density was \approx 40% less than that for the Swiss Webster, although mRNA abundance was similar in both strains. Etorphine's potency was 4-fold greater in control Swiss Webster compared to CXBK mice. Etorphine treatment decreased (\approx 25–40%) μ -receptor density similarly in both strains throughout treatment. The magnitude of analgesic tolerance to etorphine was 8-fold in both mouse strains. Etorphine produced a biphasic effect on receptor mRNA in both strains with levels decreased (25%) by 3 days and increased (30–40%) at 7 days. mRNA levels remained elevated (55%) 16 h following the end of the 7 day etorphine treatment. Taken together, these data suggest that in vivo etorphine treatment that produces μ -opioid receptor downregulation and tolerance, can regulate μ -opioid receptor mRNA abundance. Receptor downregulation may initially induce decreases in mRNA levels since downregulation preceded a decrease in gene expression. Prolonged (>3 days) receptor downregulation may be responsible for increasing message levels and may be important in recovery of receptors following treatment. In addition, the magnitude of changes in receptor density, mRNA, and tolerance were similar in both CXBK and Swiss Webster mice, indicating that the mechanisms required for receptor regulation and its functional consequences are independent of basal μ -opioid receptor density. **Synapse 37:118–124, 2000.**

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INTRODUCTION

Treatment with opioid agonists and antagonists has been shown to regulate opioid receptor density and the potency of opioid agonists. Although both opioid agonists and antagonists can regulate opioid receptor density, it appears that the mechanisms that mediate these effects differ. For example, chronic in vivo opioid antagonist treatment typically produces opioid receptor upregulation and functional supersensitivity that is not associated with changes in gene expression (e.g.,

Duttaroy et al., 1999; Jenab et al., 1995; Unterwald et al., 1995; Yoburn et al., 1993, 1995). In contrast, chronic in vivo opioid agonist treatment that produces

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receptor downregulation and tolerance can induce a change in receptor gene expression (Ronnnekleiv et al., 1996; Sehba et al., 1997). We previously showed that the high intrinsic efficacy opioid agonist etorphine produced significant downregulation and tolerance and regulated opioid receptor gene expression in vivo (Sehba et al., 1997). Specifically, μ -opioid receptor mRNA was increased following a 7-day etorphine infusion in mice and returned to control levels as μ -opioid receptor density recovered (Sehba et al., 1997). It is possible that the increase in μ -receptor mRNA may drive the recovery of receptors following chronic etorphine treatment. If the increase in μ -receptor mRNA were required for the recovery of receptors, then it might be assumed that message levels would rise soon after the termination of treatment. On the other hand, perhaps chronic opioid administration that downregulates μ -receptors is associated with an increase in receptor mRNA during the course of treatment.

To more fully explore agonist-induced regulation of μ -opioid receptor mRNA in vivo, it is important to characterize the profile of changes in receptors and mRNA during the development of downregulation and tolerance. In our previous study (Sehba et al., 1997), receptor density and mRNA were measured after the end of the 7-day etorphine treatment. Therefore, in order to develop a full profile of changes in μ -opioid receptor density and mRNA abundance, changes were measured during the 7-day etorphine infusion in the present study. In addition, to determine if basal receptor density plays a role in receptor regulation and gene expression, this study used two mouse strains (CXBK and Swiss Webster) that differ in their basal μ -opioid receptor densities in the CNS. The CXBK mouse, which is derived from a cross between the C57 and BALB strains, has significantly lower μ -opioid receptor density and μ -opioid agonists are less potent in the CXBK mouse compared to its progenitor and other outbred strains (Baran et al., 1975; Chang et al., 1998; Duttaroy et al., 1999; Moskowitz and Goodman, 1985; Pick et al., 1993). The densities of δ - and κ -opioid receptors appear to be relatively unaffected by the deficiency of μ -receptors (Baran et al., 1975; Duttaroy et al., 1999; Kest et al., 1998; Moskowitz and Goodman, 1985). In this study, standard outbred Swiss Webster mice, which have greater μ -opioid receptor density than CXBK mice, were used as a reference control (Duttaroy et al., 1999).

MATERIALS AND METHODS

Subjects

Male, CXBK mice (CXB-7/By; 20–25g; Jackson Laboratory, Bar Harbor, ME) and outbred male, Swiss-Webster mice (20–30 g; Taconic Farms, Germantown, NY) were housed 10/cage with free access to food and water. Mice were used only once.

Procedure

Mice were implanted s.c. with osmotic minipumps that infused 250 μ g/kg/day etorphine for 1, 3, 5, or 7 days. To minimize cost, controls were implanted with inert placebo pellets (Duttaroy and Yoburn, 1995). Pumps or pellets were implanted in the nape of the neck while mice were lightly anesthetized with halothane:oxygen (4:96%). One group of mice was treated for 7 days, pumps or pellets removed, and were tested for antinociception 16 h following the end of treatment (see below). For other groups of mice, μ -opioid receptor mRNA levels in whole brain were assessed by solution hybridization/ribonuclease protection assays (see below) immediately after 1, 3, 5, or 7 days treatment or 16 h after the pumps and pellets were removed on the 7th day. For other mice, pumps or pellets were removed after 1, 3, 5, or 7 days of treatment and, 16 h later, mice were sacrificed and whole brain removed for saturation binding studies (see below). The 16-h interval is required in binding assays following in vivo treatment since residual wash-resistant etorphine is retained in tissue homogenates after administration (Law et al., 1983; Tao et al., 1987; Yoburn et al., 1993). Consequently, binding cannot be accurately evaluated immediately at the end of treatment unless etorphine has been eliminated and, therefore, we conducted binding studies 16 h after the end of etorphine treatment (see Yoburn et al., 1993, for discussion).

μ -Opioid receptor saturation binding

μ -Opioid receptor saturation binding studies were performed as described previously by Yoburn et al. (1993). Mice ($n = 2$ /treatment/experiment) were sacrificed and whole brain was rapidly removed, weighed, and then homogenized in 80 volumes of ice-cold 50 mM Tris buffer (pH 7.4). Homogenates were then centrifuged at 15,000 rpm for 15 min, the supernate discarded, the pellet resuspended in buffer, the suspension centrifuged again, and the pellet frozen (-80°C) until analysis. The pellets were thawed, resuspended in Tris buffer, incubated (30 min at 25°C), centrifuged, and finally resuspended in 20 volumes of 50 mM phosphate buffer (pH 7.2). An aliquot (200 μ l) of homogenate was assayed in triplicate in tubes containing 0.03–5.0 nM [^3H] [D-Ala²-MePhe⁴-Gly(ol)⁵]enkephalin (DAMGO: μ -ligand) (Amersham Life Science, Arlington Heights, IL). Nonspecific binding was determined in the presence of 1,000 nM levorphanol. Homogenates were incubated for 90 min at 25°C . Incubation was terminated by the addition of ice-cold phosphate buffer and the samples were filtered over GF/B glass fiber filters (Brandel, Gaithersburg, MD). Filters were washed three times with cold buffer, transferred to vials, scintillation cocktail added, and then counted. Counts per minute (CPMs) were converted to disintegrations per minute (DPMs) using the external stan-

dard method. Protein was determined using a microassay technique based on the method of Bradford (1976).

Solution hybridization/ribonuclease protection assay

Total RNA was extracted from whole brain ($n = 4$ mice/treatment/experiment) as described previously (Sehba et al., 1997) using TRizol reagent (GIBCO, Gaithersburg, MD). RNA was precipitated using 100% isopropyl alcohol and washed at least twice with 75% ethanol. The pellet was dried briefly, resuspended in diethyl pyrocarbonate (Sigma, St. Louis, MO) treated water, and stored at -80°C until analyzed. The yield of each RNA extraction was determined using UV spectrophotometry (260 nm). A 668 nt antisense riboprobe directed towards mRNA for the mouse μ -opioid receptor (mMOR) was prepared (Sehba et al., 1997). To construct the mMOR riboprobe, a 668 nt PvuII-BamHI fragment from the full length cDNA (163–831 bp) (Kaufman et al., 1995) was subcloned into pGem3Zf. This fragment includes 93 bp upstream of the translation start site and coding region extending through the first six residues of transmembrane domain 4 of mMOR. The riboprobe was prepared by linearizing purified mMOR plasmid with EcoRI endonuclease and transcribing with *Sp6* RNA polymerase using [^{32}P]-labeled CTP and purified over a Sephadex G-25 column. Specific activity of the riboprobe was typically 2×10^8 CPM/ μg . To create a standard curve, mMOR plasmid was linearized with HindIII and transcribed with *T7* polymerase to produce a 668 nt (not full length) sense transcript.

Total (50 μg) RNA (25–50 μg brain + 0.25 μg yeast tRNA) was incubated and then co-precipitated with riboprobe ($\sim 2 \times 10^4$ CPMs), suspended in 20 μl of hybridization buffer (RPA II kit, Ambion, Austin, TX), allowed to hybridize for 12 h (42°C), then treated with 200 μl of 1:1000 dilution of RNase (0.5 mg/ml of RNase A; 10,000 units/ml RNase T1) for 30 min (37°C). The RNase-resistant hybrids were precipitated with 5% trichloroacetic acid and collected on glass fiber filters followed by liquid scintillation counting. A standard curve for mMOR was included in each assay using known amounts (50–500 pg) of sense transcripts (see Duttaroy et al., 2000; Sehba et al., 1997, for a representative standard curve). The values for pg of mMOR mRNA were calculated based on the 668 nt sense transcript, not the full-length transcript.

Antinociception (analgesia)

Antinociception was determined using the radiant heat tailflick method and a cumulative dose–response protocol as previously described (Duttaroy and Yoburn, 1995). Briefly, a baseline tailflick latency (2–4 sec) was determined at the end of treatment just prior to dose–response testing. Mice ($n = 8$ /treatment/experiment)

were then injected with a starting dose (0.5 $\mu\text{g}/\text{kg}$) of etorphine and tested 15 min postinjection. Mice that had a tailflick latency of 10 sec were defined as antinociceptive (i.e., analgesic). Mice that were not analgesic were injected with another dose (i.e., increment dose) of etorphine and retested 15 min later (increment dose range 1–200 $\mu\text{g}/\text{kg}$). Cumulative dosing was continued until all mice were analgesic (cumulative dose range 0.5–570 $\mu\text{g}/\text{kg}$). Mice were tested by an observer who was not aware of the pretreatment group assignment.

Drugs

Etorphine HCl and placebo pellets were obtained from Research Triangle Institute (Research Triangle Park, NC) through the Research Technology Branch of the National Institute on Drug Abuse. All pellets were wrapped in nylon mesh before s.c. implantation in the nape of the neck. For the dose–response study, etorphine was dissolved in 0.9% NaCl and doses expressed as the base.

Data analysis

Quantal dose–response data were analyzed by Probit Analysis (Finney, 1973) which estimates ED_{50} s, 95% confidence limits, and relative potencies. Statistical differences between ED_{50} values were determined using significant differences for the relative potency estimates ($P < 0.05$) as determined by Probit Analysis. Saturation binding data were used to estimate B_{max} and K_d parameters using nonlinear regression (Prism ver. 1.03; Graphpad Software, San Diego, CA) for individual experiments. In all cases, binding data were fit best by a one-site model. Differences between groups in replicate binding and mRNA experiments were analyzed using one-way ANOVA and appropriate post-hoc *t*-tests with Bonferroni's correction.

RESULTS

In control (placebo-treated) mice, basal μ -opioid receptor density was significantly ($P < 0.05$) less (-41%) in CXBK mouse brain compared to Swiss Webster mouse brain (mean \pm SEM B_{max} from 16 separate experiments = 222 ± 20 ; 132 ± 11 fm/mg of protein; CXBK-Placebo, Swiss Webster-Placebo, respectively). Receptor affinity did not differ significantly ($P > 0.05$) for control mice (mean \pm SEM K_D values from 16 separate experiments = 1.1 ± 0.3 , 0.9 ± 0.2 nM; CXBK-Placebo, Swiss Webster-Placebo, respectively). The abundance of basal μ -opioid receptor mRNA in whole brain was similar ($P > 0.05$) in control mice of both strains (mean \pm SEM mMOR mRNA from 14 separate experiments = 10.2 ± 0.9 , 10.8 ± 0.7 pg/ μg of total mRNA; CXBK-Placebo, Swiss Webster-Placebo, respectively). In control Swiss Webster mice, etorphine's potency was 4.4-fold greater than in CXBK mice (Table I).

TABLE I. The effect of chronic etorphine on the analgesic potency of etorphine

Treatment	Etorphine ED ₅₀ (mg/kg)	ED ₅₀ shift
<i>Swiss Webster</i>		
Placebo	4.9 (3.3–7.3)	
Etorphine	39.5 (28.0–55.9)	8*
<i>CXBK</i>		
Placebo	21.5 (13.6–33.3)	
Etorphine	167.7 (110.9–243.3)	8*

Mice (CXBK, Swiss Webster) were implanted with placebo pellets or osmotic minipumps that infused 250 μg/kg/day etorphine for 7 days, pumps and pellets removed and 16 hr later s.c. etorphine analgesia was measured in a cumulative dose-response study. ED₅₀ ± 95% CL are from one of two experiments that yielded similar results. (ED₅₀ shift = ED₅₀ for placebo/ED₅₀ for naltrexone). **P* < 0.05 significantly different from placebo by Probit Analysis (see Methods).

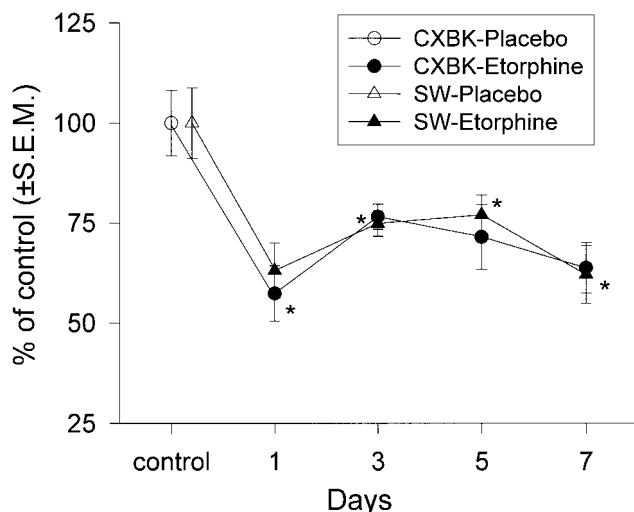


Fig. 1. The effect of etorphine on mouse brain μ-opioid receptor binding. CXBK and Swiss Webster (SW) mice were implanted with placebo pellets or osmotic minipumps that infused 250 μg/kg/day etorphine for 1, 3, 5, or 7 days. Pumps and pellets were removed and 16 h later whole-brain saturation binding studies were conducted with [³H]DAMGO. The B_{max} data were converted to percent of control values for each experiment. The B_{max} (means ± SEM) in mouse brain for all controls were 222 ± 20 and 132 ± 11 fm/mg of protein for CXBK-Placebo and Swiss Webster-Placebo, respectively. Data are means ± SEM from 3–5 individual experiments for each time point. **P* < 0.05 significantly different from corresponding control by post-hoc tests (see Methods).

Etorphine treatment significantly (*P* < 0.05) decreased (≈25–40%) μ-opioid receptor B_{max} in both CXBK and Swiss Webster mice (Fig. 1) at days 1, 3, 5, and 7. The percent decreases in receptor density were similar for both strains (Fig. 1). Receptor affinity was not significantly (*P* > 0.05) changed by etorphine treatment at any time point examined.

Etorphine treatment produced a biphasic effect on CXBK and Swiss Webster mouse brain μ-opioid receptor mRNA levels (Fig. 2). One-day etorphine treatment did not produce any significant change (*P* > 0.05), while the 3-day treatment significantly (*P* < 0.05) decreased (25%) mMOR mRNA levels in both strains. The 5-day etorphine infusion produced no change in (*P* > 0.05), and the 7-day treatment significantly (*P* < 0.05) increased (30–40%) the mMOR mRNA level. mMOR

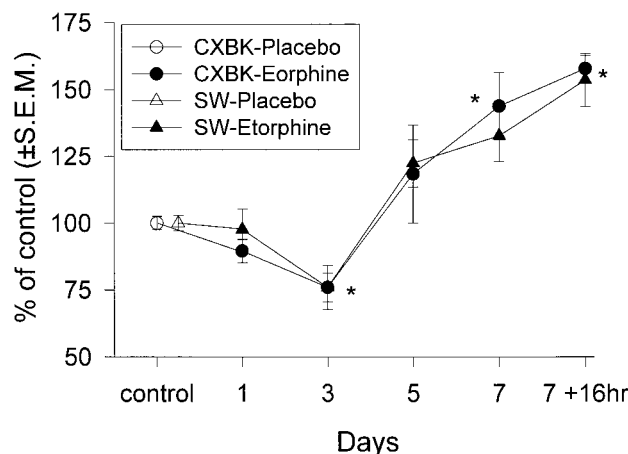


Fig. 2. The effect of etorphine on mouse brain μ-opioid receptor (mMOR) mRNA. CXBK and Swiss Webster (SW) mice were implanted with placebo pellets or osmotic minipumps that infused 250 μg/kg/day etorphine for 1, 3, 5, or 7 days. Immediately after treatment or 16 h following removal of the pumps and pellets at 7 days (7 + 16 hr), mouse whole brain was removed, total RNA extracted, and mMOR mRNA was determined by solution hybridization/ribonuclease protection assay. The mMOR mRNA data were converted to percent of control values for each experiment (mean ± SEM mMOR mRNA in mouse brain for all controls were 10.2 ± 0.85 and 10.8 ± 0.71 pg/μg total RNA for CXBK-Placebo and Swiss Webster-Placebo, respectively). Data are means (±SEM) from 2–3 individual experiments for each time point. **P* < 0.05 significantly different from corresponding placebo-treated mice by post-hoc tests (see Methods).

mRNA levels remained elevated (55%; *P* < 0.05) 16 h following the end of 7-day etorphine treatment. The magnitude and the direction of changes in mMOR mRNA levels were virtually identical in CXBK and Swiss Webster mice.

Chronic 7-day etorphine treatment significantly (*P* < 0.05) decreased the potency of etorphine (8-fold) in both CXBK and Swiss Webster mice (Fig. 3; Table I). There were no significant differences (*P* > 0.05) among the groups in baseline tailflick latencies determined prior to testing.

DISCUSSION

In the present study, chronic in vivo treatment with the high intrinsic efficacy opioid agonist etorphine decreased μ-opioid receptor density, induced tolerance, and simultaneously regulated μ-opioid receptor mRNA. These data extend previous findings from our lab that demonstrated that μ-opioid receptor mRNA levels are elevated following the end of a 7-day etorphine treatment that produces receptor downregulation and tolerance (Sehba et al., 1997). Taken together, the results of these studies indicate etorphine treatment can both decrease and increase μ-opioid receptor mRNA in vivo. During etorphine treatment, mRNA levels initially decline and then are elevated with continued treatment. When etorphine treatment is terminated, mRNA abundance in brain eventually declines to control levels as μ-receptor density recovers (Sehba et al., 1997). Even though etorphine biphasically regu-

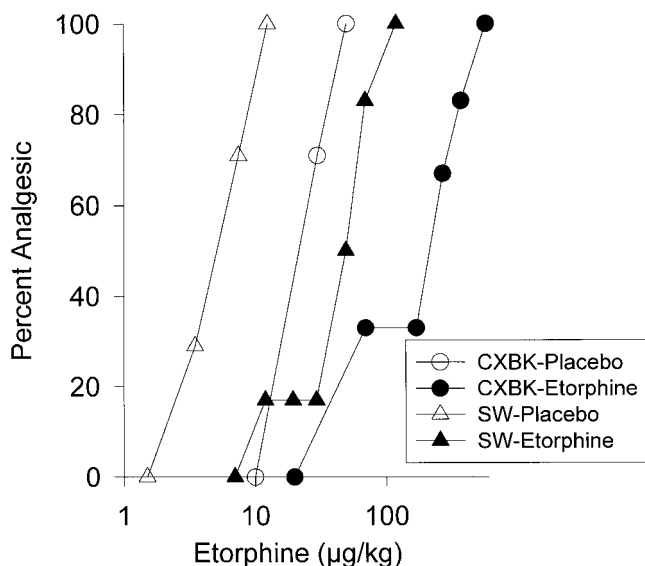


Fig. 3. The effect of chronic etorphine on the analgesic potency of etorphine. CXBK and Swiss Webster (SW) mice were implanted with placebo pellets or osmotic minipumps that infused 250 µg/kg/day etorphine for 7 days, pumps and pellets removed, and 16 h later s.c. etorphine analgesia was evaluated in a cumulative dose-response study. Data are from one of two experiments showing similar results.

lated μ -opioid receptor mRNA in the present study, receptor density remained decreased throughout the etorphine infusion.

The initial decline in μ -opioid receptor mRNA might be a consequence of μ -receptor downregulation. The fact that a decrease in message was not observed until day 3, while receptor downregulation was noted earlier (i.e., following 24 h of etorphine treatment), supports this suggestion. Downregulation of μ -receptors was relatively constant throughout the etorphine infusion. In contrast, receptor message levels were increased by day 7 of etorphine treatment. Perhaps the increase in message is induced by prolonged downregulation in addition to the development of tolerance, which together may serve as stimuli to increase μ -opioid receptor gene expression. It should be noted that tolerance to etorphine was measured only at the end of the 7-day treatment and, therefore, it is not clear at what point significant tolerance had developed. However, preliminary data from our lab have shown that tolerance to etorphine (\approx 6-fold) is present within 48 h of the start of an etorphine infusion. In any case, although mRNA levels increased by day 7, receptor density remained significantly decreased, suggesting that the increase in message was insufficient to overcome the profound downregulatory effects of etorphine. However, as we have shown previously (Sehba et al., 1997), when the etorphine infusion is terminated, μ -receptor density recovers over the course of 7 days in concert with a decline in receptor mRNA levels. Taken together, these data suggest that the increase in μ -opioid receptor mRNA may be required for the recovery of μ -receptor

density at the end of treatment. Furthermore, it might be the case that if receptor mRNA levels were not increased during treatment that downregulation might be even more substantial.

The onset of *in vivo* downregulation within 24 h might have been predicted based on the rapid time course of μ -receptor internalization (Chu et al., 1997; Keith et al., 1998; Sternini et al., 1996; Whistler and von Zastrow, 1998; Zhang et al., 1998). Confocal imaging studies in brain neurons indicate that etorphine produces naloxone reversible μ -receptor internalization within 30 min of treatment (Keith et al., 1998; Sternini et al., 1996). Continued internalization and agonist-receptor occupancy may induce trafficking of receptors to the lysosomal pathway and subsequent downregulation (Chakrabarti et al., 1997; Keith et al., 1996; Krupnick and Benovic, 1998; Roth et al., 1998). However, internalization and downregulation during the first 24 h of treatment in this study did not serve as an adequate stimulus for regulation of μ -opioid receptor mRNA, since changes were not observed until 3 days later. Our data agree with a previous cell culture study (Kim et al., 1995) in which etorphine-induced downregulation of δ -opioid receptor density occurred within 1 day, but a decrease in δ -receptor mRNA levels requires 3–5 days.

It is possible that a prolonged period of etorphine exposure produces an initial overstimulation of the signal transduction pathways and this sets the occasion for regulation of receptor density and mRNA. Specifically, etorphine induces rapid internalization followed by the development of downregulation and eventually a reduction in receptor mRNA. In addition, it is likely that the remaining complement of μ -receptors become desensitized as a result of phosphorylation (Pei et al., 1995; Yu et al., 1997; Zhang et al., 1998). Initially, these responses to etorphine represent an attempt to return the signaling pathways to the basal state. However, with the cumulative consequences of desensitization, and as more receptors are lost to downregulation, there may be a compensatory response that results in synthesis of new receptors. The synthesis of new receptors would require an increase in receptor gene expression, as observed in the present study. Thus, while receptors were still downregulated by etorphine at this point, the processes that are required for the recovery of receptors may have commenced. The increase in μ -receptor mRNA levels may ultimately drive the recovery of receptors (Sehba et al., 1997). However, not all increases in opioid receptor density require a change in receptor gene expression. As noted previously, *in vivo* opioid antagonist-induced receptor upregulation does not produce an increase in receptor mRNA (Castelli et al., 1997; Duttaroy et al., 1999; Unterwald et al., 1995). Furthermore, recovery of μ -opioid receptor density following depletion by alkylation was not associated with any increases in mRNA

levels (Chan et al., 1997). These data support suggestions that there are multiple mechanisms for regulation of opioid receptors.

Previous studies have shown that biphasic changes in receptor density and mRNA can occur in other neuronal pathways following the end of chronic drug treatment. For example, both increases and decreases have been observed in dopaminergic receptor densities and mRNA abundance in select brain areas in rat following withdrawal from cocaine treatment (Kleven et al., 1990; Laurier et al., 1994). Taken together with the present study, one can conclude that biphasic changes in receptor mRNA can be induced not only from drug withdrawal but also during drug treatment.

Previous studies have indicated that residual etorphine may interfere with accurate estimates of parameters in receptor binding studies (Law et al., 1983; Tao et al., 1987; Yoburn et al., 1993). However, if a 16-h interval is employed following in vivo etorphine exposure, residual drug bound to membranes is eliminated (Yoburn et al., 1993). It might be suggested that this interval complicates interpretation, since some recovery from receptor downregulation may have occurred. While this might be the case, it is clear that downregulation is still present and that tolerance can be readily measured. We anticipated that changes in mRNA levels precede changes in receptor density and, therefore, assessed the time course of changes in mRNA following 1, 3, 5, and 7 days of etorphine treatment without the 16-h interval. To confirm that the interval does not affect the measurement of mRNA, changes in μ -receptor mRNA were also determined 16 h following the end of the 7-day etorphine infusion. Our results show that with and without the 16-h interval that μ -receptor mRNA levels were increased similarly after the 7-day infusion. Consequently, we conclude that the interval is not a critical factor for assessing changes in mRNA levels.

We observed no difference in the effects of etorphine in the two strains of mice used in this study. Etorphine produced similar μ -receptor downregulation and tolerance in both the CXBK and Swiss Webster mice. Furthermore, etorphine treatment produced a comparable pattern of changes in μ -opioid receptor mRNA levels in both mouse strains. Thus, there was no effect of basal μ -opioid receptor complement on opioid receptor regulation and tolerance. These data support the results of a previous study on μ -opioid receptor regulation by opioid antagonists that demonstrated that basal receptor density had no effect on receptor upregulation or supersensitivity (Duttaroy et al., 1999). Thus, it appears that all of the regulatory mechanisms for μ -receptors are functional in the μ -receptor-deficient CXBK mouse. As we reported previously, basal mRNA levels do not differ between the CXBK and Swiss Webster mice (Duttaroy et al., 1999).

In summary, the present study evaluated the profile of opioid agonist-induced μ -opioid receptor regulation in vivo. Etorphine treatment downregulated μ -opioid receptor density, produced tolerance, and induced an initial decrease in receptor mRNA level. The initial decrease in message level was followed by an increase, and it is likely that this increase in gene expression initiates and eventually drives the recovery of receptors at the end of etorphine treatment, as suggested previously (Sehba et al., 1997). Finally, a deficit in μ -opioid receptor numbers had no impact on the magnitude of regulatory effects of chronic opioid agonist treatment in vivo. Therefore, the magnitude of dynamic changes in receptors and opioid potency are independent of basal receptor density.

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