OPIOID RECEPTOR UPREGULATION IN μ-OPIOID RECEPTOR DEFICIENT CXBK AND OUTBRED SWISS WEBSTER MICE

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(Received in final form March 17, 1999)

Summary

Chronic in vivo treatment with opioid antagonists increases opioid receptor density and the potency of opioid agonists without altering receptor mRNA levels. To determine if basal receptor density affects opioid receptor upregulation, we examined the effect of chronic naltrexone treatment on μ-opioid receptor density and mRNA in two mice strains that differ in μ-opioid receptor density. CXBK mice (μ-opioid receptor deficient) and outbred Swiss Webster mice were implanted s.c. with a placebo or 15 mg naltrexone pellet for 8 days, the pellets removed and 24 hr later opioid receptor density (μ, δ) and receptor mRNA level (μ) determined in whole brain; or morphine dose-response studies conducted. In placebo-treated CXBK mice, μ-opioid receptor density was ≈40% less than in Swiss Webster mice, although μ-opioid receptor mRNA abundance was similar in both strains. In placebo-treated CXBK mice, morphine potency was ≈6-fold less than Swiss Webster mice. Naltrexone treatment increased morphine potency (1.7-fold) and μ-(≈90%) and δ- (≈20-40%) opioid receptor density in CXBK and Swiss Webster mouse brain similarly. μ-opioid receptor mRNA was unchanged by naltrexone treatment in either strain. There was no difference in the basal or naltrexone-treated whole brain G102 protein levels in CXBK or Swiss Webster mouse. These data indicate that a deficiency in μ-opioid receptors does not alter the regulation of opioid receptors by opioid agonists in vivo, and suggest that adaptive responses to chronic opioid antagonist treatment are independent of opioid receptor density.

Key Words: μ-opioid receptor, δ-opioid receptor, μ-receptor mRNA, G102, protein, upregulation, naltrexone, morphine, CXBK mice, Swiss Webster mice

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Treatment with opioid agonists or antagonists can regulate opioid receptor density as well as the potency of opioid agonists. For example, chronic in vivo opioid antagonist (e.g., naltrexone) treatment will increase the density of opioid receptors (i.e., upregulation) and produce a parallel increase in the potency of opioid agonists (i.e., functional supersensitivity) (e.g., 1-4). While the density of μ-, δ- and κ-opioid receptors reliably increases after chronic opioid antagonist exposure in vivo, the origin of the new receptors is not clear, since a concurrent change in in vivo receptor gene expression (i.e., steady state mRNA level) has not been observed (5-8). Consistent with these results, an antisense oligodeoxynucleotide to the μ-opioid receptor does not block opioid antagonist-induced μ-receptor upregulation or supersensitivity (9) and an in vitro study showed that antagonist-induced upregulation was not blocked by cyclohexamide (10). Overall, the fact that opioid antagonists increase opioid receptor number in vivo without a change in receptor mRNA levels suggests that increases in receptor density may not require the synthesis of new receptors. It is possible that opioid antagonist-induced upregulation may be due activation of a pre-existing pool of receptors (i.e., 'cryptic' receptors) that become detectable in binding studies (11,12,13) or a change in degradation (14), translation (10) or cycling (15) of receptors. This raises the possibility of several different mechanisms that might account for opioid antagonist-induced receptor upregulation in vivo.

Although opioid receptor number can be increased by antagonist treatment in vivo and does not appear to involve changes in gene expression, it is not clear if this would also be the case if there were an initial deficit in receptor number. It might be predicted that a deficit in opioid receptor number would favor an increase in receptor mRNA following antagonist-induced upregulation, since synthesis of new receptors might be required to increase receptor density. An often-used model of μ-opioid receptor deficiency is the CXBK mouse. The CXBK mouse, which is derived from a cross between the C57 and BALB strains, has significantly reduced density of μ-opioid receptors compared to its progenitor strains, particularly in areas involving pain processing (16,17). The density of δ- and κ-opioid receptors appears to be relatively unaffected by the deficiency in μ-opioid receptors (16,17). As might be predicted based on receptor theory (18), μ-opioid agonists are significantly less potent in the CXBK mouse compared to their progenitor strains and other outbred mice strains (e.g., 16,17,19-21).

In the present study, the effect of chronic opioid antagonist treatment on μ-and δ-opioid receptor density, μ-opioid receptor mRNA and changes in opioid agonist potency were evaluated in the CXBK and Swiss Webster mouse strains. We hypothesized that the deficiency in μ-opioid receptors in the CXBK might result in opioid antagonist-induced increases in μ-opioid receptor density as well as increases in gene expression since synthesis of new receptors might be required. Furthermore, since studies have demonstrated that μ-opioid receptors are functionally coupled to signaling mechanisms via $G_{μ2}$ proteins (22-25), we examined if the reduced potency of opioids in the CXBK mouse was associated with a change in the level of this G-protein in brain.

**Methods**

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

*Naltrexone Treatment.* Mice were implanted s.c. with an inert placebo or a 15 mg naltrexone pellet for 8 days. Pellets were implanted in the nape of the neck while mice were under light anesthesia with halothane:oxygen (4%:96%). Pellets were removed after treatment and 24 hr later mice (N=2-4/treatment) were sacrificed and whole brain removed for saturation binding studies, solution hybridization/ ribonuclease protection assays, or for Western blot analysis (see below).
Other mice (N=8/treatment), were treated similarly and were tested for antinociception 24 hr following pellet removal (see below).

Antinociception (Analgesia). Antinociception was determined using the radiant heat tailflick method and a cumulative dose-response protocol as previously described (26). Briefly, a baseline tailflick latency (2-4 sec) was determined at the end of treatment prior to dose-response testing. Mice were then injected s.c. with a starting dose (0.5 mg/kg) of morphine and tested 30 min post-injection. Mice that were not analgesic were injected with another dose (i.e., increment dose) of morphine and retested 30 min later (increment dose range = 1-10 mg/kg). Cumulative dosing was continued until all mice were analgesic. Mice were tested by an observer who was not aware of the pretreatment group. The dose-response data for Swiss Webster mice presented in this paper are from Shah et al. (9) using an identical treatment protocol. Chronic naltrexone-induced supersensitivity to the analgesic potency of morphine in Swiss Webster mice is a well-established phenomenon, and previous studies have shown that 8-day naltrexone treatment produces ~2-fold shift in the \( ED_{50} \) for morphine (3,9,27,28).

\( \mu \) - and \( \delta \)-opioid receptor binding assay. Opioid receptor binding studies were performed as described previously (29). Briefly, mice (N= 2/treatment) were sacrificed and whole brain was rapidly removed, weighed and then homogenized in ice cold 50 mM Tris buffer (pH 7.4). Homogenates were centrifuged twice, the pellet resuspended in buffer, and 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 of homogenate was assayed in triplicate in tubes containing 0.03-5.0 nM \(^{3}H\) [D-Ala\(^2\)-MePhe\(^4\)-Gly(ol)\(^5\)]enkephalin (DAMGO: \( \mu \)-ligand; specific activity = 54 Ci/mmol) (Amersham Life Science, Arlington Heights, IL), 0.03-6.0 nM \(^{3}H\) [D-Pen\(^2\),D-Pen\(^3\)]enkephalin (DPEPE; \( \delta \)-ligand; specific activity = 32 Ci/mmol) or 0.03-5.0 nM \(^{3}H\)Deltophin II (\( \delta \)-ligand, specific activity = 54.5 Ci/mmol) (New England Nuclear, Boston, MA). Nonspecific binding was determined in the presence of 1000 nM levorphanol. Homogenates were incubated for 90 min at 25°C and then 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 disintegration per minute (DPMs) using the external standard method. Protein was determined using a microassay technique based on the method of Bradford (30).

Solution hybridization/ribonuclease protection assay. Total RNA was extracted from whole brain (N= 3-4 mice/treatment/ experiment) as described previously (31) using TRizol reagent (GIBCO BRL, Gaithersburg, MD). RNA was precipitated using 100% isopropyl alcohol, washed with 75% ethanol, the pellet dried and resuspended in diethyl pyrocarbonate treated water and stored (-80°C). The yield for each RNA extraction was determined using UV spectrophotometry (260 nm). A 668 nt mouse \( \mu \)-opioid receptor (mMOR) riboprobe was constructed from the full length cDNA (see 32) as previously described (31). To construct a mMOR riboprobe, a 668 nt (163-831 bp) PvuII - BamHI fragment from the full length cDNA was subcloned into pGem3zf. This fragment includes 93 bp upstream of the translation start site and coding region extending through the first 6 residues of transmembrane domain 4 of mMOR. The riboprobe is prepared by linearizing purified mMOR plasmid with EcoRI endonuclease and transcribing with \( S\phi 6 \) RNA polymerase using \(^{3}P\)-labeled CTP and purified over a Sephadex G-25 column. A single 668 nt band was observed when an aliquot of riboprobe was analyzed by urea-PAGE and autoradiography (31). Specific activity of the riboprobe was typically 2 x10\(^{6}\) CPM/\mu g. In addition, mMOR plasmid was linearized with HindIII and transcribed with T7 polymerase to produce a 668 nt (not full-length) sense transcript for use in standard curve construction.

Total (50 \( \mu \)g) RNA (25-50 \( \mu \)g brain + 0-25 \( \mu \)g yeast tRNA) and the riboprobe (~2 x 10\(^{4}\) CPMs) were incubated, co-precipitated and then suspended in 20 \( \mu \)l of hybridization buffer (RPA II kit,
Ambion, Austin, TX), allowed to hybridize for 12 hr (42°C) and 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. The values for pg of mMOR mRNA were calculated from the standard curve using the 668 nt sense transcript. A representative standard curve is shown in Fig. 1. A linear relationship between sense and CPMs was observed in all assays (mean±S.E.M. r² = 0.95±0.01). The values for pg of mMOR mRNA reported in this paper were calculated based on the 668 nt sense transcript, not the full length transcript.

![Fig. 1](image)

A typical standard curve for mouse μ-opioid receptor (mMOR) mRNA. The mMOR plasmid was linearized with HindIII and transcribed with T7 polymerase to produce a 668nt sense transcript (not full length). Known amounts (50 – 500 pg) of sense transcripts were included in each assay and subjected to the same ribonuclease protection procedure as RNA extracted from mouse brain. A standard curve was included in each assay. Specific hybridization is measured in CPMs for each sense concentration, which represents the TCA precipitated hybrids collected on filters minus background (yeast tRNA alone) CPMs. A linear relationship between sense and CPMs was observed in all assays. A representative curve from one of three experiments is shown.

To examine the specificity of the effect of naltrexone on mMOR mRNA, we also estimated the changes in glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA which is a standard house-keeping gene used in previous studies (5, 7, 31). To construct the GAPDH antisense riboprobe (316 nt), linearized plasmids containing rat GAPDH sequence were purchased (Ambion, Austin, TX) and transcribed by T7 polymerase as previously described (31). The specific activity of the probe was typically 2 x 10⁶ cpm/μg. For GAPDH, a sense curve was not constructed, however, the slope of the relationship between varying total RNA concentration and CPMs for the GAPDH riboprobe was used to calculate percent of control for each experiment (31).

**Immunoblot analysis of G1a2.** Mice (N= 2/ treatment/ experiment) were sacrificed and whole brain removed, suspended in ice-cold TME buffer (50 mM Tris-HCl, pH 7.4, 2 mM MgCl₂, 0.3 mM EGTA), homogenized and then centrifuged and the pellet frozen (-80°C) until analysis. Western analysis was conducted as previously described (25). Briefly, samples were separated on 10% polyacrylamide minigels (Bio-Rad, Richmond, CA) and transferred to nitrocellulose membranes. Membranes were then incubated with anti mouse G1a2 antibody (Chemicon, Temecula, CA) washed, and incubated with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (Amersham, Arlington Heights, IL). The blots were visualized using an Enhanced Chemiluminescence (ECL) western blot detection reagent kit and x-ray film (Amersham, Arlington Heights, IL). The blots were digitized and analyzed for optical density with Gel-Pro image analysis software (ver. 3.0, Media Cybernetics, Silver Spring, MD). A standard curve using
known amounts (20-50 ng) of recombinant mouse G_{i/o2} protein (Chemicon, Temecula, CA) was included in each gel assay as described earlier (25). Typical standard curves were linear over the entire range of known amounts (mean±S.E.M. \( r^2 = 0.97±0.05 \), 3 experiments; see 25 for representative curve).

**Drugs.** Naltrexone and corresponding 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 of the mouse. Morphine sulfate (Penick Corporation, Newark, NJ) was dissolved in 0.9% NaCl and doses expressed as the base.

**Data analysis.** Quantal dose-response data were analyzed by Probit Analysis (33) which estimates ED_{50}s, 95% confidence limits and relative potencies. Significant differences between ED_{50}s were determined using Probit Analysis. \( B_{max} \) and \( K_d \) estimates were determined from saturation studies using nonlinear regression (Prism ver 1.03, Graphpad Software, San Diego, CA). In all cases, binding data were fit best by a one-site model. Differences between groups for data from binding, mRNA and immunoblot studies were analyzed using ANOVA and appropriate post hoc t-tests. All mRNA data were converted to percent of control prior to statistical analysis.

**Results**

Saturation binding studies using \([^3]H\)DAMGO showed that basal (placebo-treated) μ-opioid receptor density was significantly (\( p<0.05 \)) less (-42%) in CXBK mouse brain homogenates compared to Swiss Webster mouse brain (Fig 2 left panel; Table I). The deficit in μ-receptors in the CXBK was relatively selective with no significant differences (\( p>0.05 \)) in the density of δ₁- or δ₁-opioid receptors (Fig 2 center and right panels; Table I). Receptor affinity did not differ significantly (\( p>0.05 \)) between the strains (Table I).

![Graphs showing binding studies](image)

**Fig. 2**

The effect of chronic naltrexone (NTX) on mouse brain μ (DAMGO), δ₂ (Deltorphin II) and δ₁ (DPDPE) opioid receptor binding. Mice (CXBK and Swiss Webster) were implanted with a placebo (Pla) or 15 mg NTX pellet as described in Methods. Data are from one representative experiment for each ligand.

Chronic naltrexone treatment significantly (\( p<0.05 \)) increased μ-, δ₁-, and δ₂-opioid receptor \( B_{max} \) in CXBK and Swiss Webster mice (Fig. 2, Table I). The percent increases in receptor density for both strains were similar for the μ-ligand (~90%), and for the δ₂-ligand \([^3]H\)Deltorphin II (37%).
Naltrexone treatment significantly increased the density of sites labeled with \(^{3}H\)DPDPE (\(\delta\)-ligand) in both strains. The increase in \(\delta\)_sites for the CXBK mice (20%) was less but not significantly different from that for the Swiss Webster mice (39%). Naltrexone treatment did not produce any significant (\(p > 0.05\)) changes in \(K_d\) for any ligand tested (Table I).

**TABLE I**

The effect of chronic naltrexone on mouse brain opioid receptor binding.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(B_{\text{max}}) (fmol/mg protein)</th>
<th>% Change in (B_{\text{max}}) (relative to Placebo)</th>
<th>% of Swiss Webster (B_{\text{max}}) (relative to corresponding treatment group)</th>
<th>(K_d) (nM)</th>
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</thead>
<tbody>
<tr>
<td>(^{3}H)DAMGO</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Swiss Webster-Placebo</td>
<td>171±2</td>
<td>-</td>
<td>-</td>
<td>0.6±0.0</td>
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<tr>
<td>Swiss Webster-NTX</td>
<td>331±23</td>
<td>94%(^*)</td>
<td>-</td>
<td>0.7±0.1</td>
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<tr>
<td>CXBK-Placebo</td>
<td>99±7</td>
<td>-</td>
<td>-22%(^\delta)</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>CXBK-NTX</td>
<td>185±11</td>
<td>87%(^*)</td>
<td>-44%(^\delta)</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>(^{3}H)Deltorphin II</td>
<td></td>
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</tr>
<tr>
<td>Swiss Webster-Placebo</td>
<td>128±24</td>
<td>-</td>
<td>-</td>
<td>0.7±0.2</td>
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<tr>
<td>Swiss Webster-NTX</td>
<td>175±9</td>
<td>37%(^*)</td>
<td>-</td>
<td>0.8±0.1</td>
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<td>CXBK-Placebo</td>
<td>123±9</td>
<td>-</td>
<td>-1%</td>
<td>0.7±0.0</td>
</tr>
<tr>
<td>CXBK-NTX</td>
<td>169±10</td>
<td>37%(^*)</td>
<td>-1%</td>
<td>0.7±0.0</td>
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<tr>
<td>(^{3}H)DPDPE</td>
<td></td>
<td></td>
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<tr>
<td>Swiss Webster-Placebo</td>
<td>126±8</td>
<td>-</td>
<td>-</td>
<td>3.6±1.3</td>
</tr>
<tr>
<td>Swiss Webster-NTX</td>
<td>175±11</td>
<td>39%(^*)</td>
<td>-</td>
<td>2.7±0.2</td>
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<tr>
<td>CXBK-Placebo</td>
<td>138±7</td>
<td>-</td>
<td>+1%</td>
<td>2.1±0.4</td>
</tr>
<tr>
<td>CXBK-NTX</td>
<td>165±4</td>
<td>20%(^*)</td>
<td>+1%</td>
<td>2.1±0.4</td>
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Mice (CXBK and Swiss Webster) were treated with placebo or 15 mg naltrexone (NTX) pellets as described in the Methods. Data are mean (±S.E.M.) from 3 separate experiments. The \(^{3}H\)DPDPE data for CXBK are presented as the mean \(B_{\text{max}}\) and \(K_d\) and respective S.E.M. from 2 individual experiments. \(^*p<0.05\) significantly different from corresponding placebo-treated mice. \(^\delta p<0.05\) significantly different from corresponding Swiss Webster group.

Whole brain mouse \(\mu\)-opioid receptor (mMOR) mRNA was similar (\(p>0.05\)) in placebo-treated CXBK (3.8±0.9 pg/\(\mu\)g total RNA) and Swiss Webster mice (4.0±0.5 pg/\(\mu\)g total RNA). mMOR mRNA in brain was not altered (\(p>0.05\)) by chronic naltrexone treatment in either CXBK or Swiss Webster mice (Fig 3). Similarly, there was no significant effect (\(p>0.05\)) of naltrexone treatment on GAPDH mRNA in both CXBK and Swiss Webster mice (Fig 3).

Morphine was significantly (\(p<0.05\)) less potent in placebo-treated CXBK mice compared to Swiss Webster mice. The \(ED_{50}\) was =6-fold higher in both placebo-treated and naltrexone-treated CXBK mice compared to the corresponding Swiss Webster strain (Fig 4). Chronic opioid antagonist treatment significantly (\(p<0.05\)) increased the potency of morphine (1.7-fold) in both CXBK and Swiss Webster mice. There were no significant differences (\(p<0.05\)) in the baseline tailflick latencies (mean±S.E.M.) among all groups (1.8±0.1, 1.6±0.1, 2.3±0.1, 2.4±0.2; CXBK-Placebo, CXBK-Naltrexone, Swiss Webster-Placebo, Swiss Webster-Naltrexone; respectively).
The effect of chronic naltrexone (NTX) on mouse brain μ-opioid receptor (mMOR) and GAPDH mRNA. Mice (CXBK and Swiss Webster) were treated with placebo (Pla) or 15 mg NTX pellets as described in the Methods. The mMOR mRNA data were converted to percent of control values for each experiment (mean±S.E.M. mMOR mRNA in mouse brain = 3.8±0.9, 3.7±0.6, 4.0±0.5, 3.7±0.3 pg/μg total RNA; CXBK-Pla, CXBK-NTX, Swiss Webster-Pla, Swiss Webster-NTX; respectively). Pg/μg of total RNA is the value determined from the 668 nt mMOR mRNA sense transcript. For GAPDH, the slope of the relationship between varying total RNA concentration and CPMs for the GAPDH riboprobe was used to calculate percent of control for each experiment (31). Data are mean (±S.E.M.) from three individual experiments.

The effect of chronic naltrexone (NTX) on s.c. morphine’s analgesic potency. Mice (CXBK and Swiss Webster) were treated with placebo (Pla) or 15 mg NTX pellets as described in the Methods. ED$_{50}$ (±95% CL) = 21.2 (16.2-26.3), 12.5 (9.1-16.6)*, 3.2 (2.6-3.8), 1.9 (1.5-2.3)*; CXBK-Pla, CXBK-NTX, Swiss Webster-Pla, Swiss Webster-NTX; respectively. Data are from one representative experiment. Swiss Webster data are from Shah et al., 1997. *p<0.05 significantly different from corresponding placebo-treated mice.
G_{ia2} protein levels were determined using Western immunoblot assays in CXBK and Swiss Webster mouse whole brain preparations. There were no significant differences (p>0.05) in the levels of G_{ia2} between CXBK and Swiss Webster mice; nor was there any effect of naltrexone treatment on either strain (G_{ia2} protein levels = 0.61±0.11, 0.60±0.08, 0.57±0.11, 0.64±0.06 ng/μg total protein; CXBK-Placebo, CXBK-Naltrexone, Swiss Webster-Placebo, Swiss Webster-Naltrexone; respectively; mean±S.E.M. from three individual experiments).

Discussion

In this study, the effects of in vivo opioid antagonist treatment on opioid receptor density, gene expression and the functional potency of morphine in two strains of mice were examined. Chronic opioid antagonist treatment produced upregulation of µ- and δ-opioid receptors and functional supersensitivity to morphine in both the CXBK and Swiss Webster mouse. Although, opioid antagonist treatment increased receptor density and morphine potency, there was no change in µ-opioid receptor mRNA in mouse whole brain. These findings confirm, and extend to the mouse, previous in vivo studies in the rat that have shown that opioid antagonist-induced increases in opioid receptor density are not associated with increases in receptor mRNA (6-8, 19).

Although the density of µ-opioid receptors in the CXBK mouse was significantly less compared to the Swiss Webster mouse, the abundance of µ-opioid receptor mRNA was similar in both strains. It is possible that the difference in receptor levels may be accounted for by a more rapid receptor degradation rate for the CXBK, or perhaps different translation rates in the two strains. Interestingly, a previous study has shown that the CXBK mouse has lower levels of mRNA in select brain regions compared to the outbred CD-1 mouse (19). The reason for the discrepancy in results may be due to differences in the outbred reference mouse strains that were employed (i.e., CD-1 vs. Swiss Webster). Although we did not find any differences in mRNA levels in the CXBK and Swiss Webster strains, it is possible that examination of select brain areas may have revealed differences in mRNA abundance between the CXBK and Swiss Webster mouse.

Chronic opioid antagonist treatment produced similar upregulation of µ- and δ-opioid receptors in the CXBK and Swiss Webster mouse. The magnitude of upregulation of δ receptors was somewhat less in the CXBK mouse. It was possible that upregulation of µ-opioid receptors in the CXBK mouse would require different mechanisms than in the Swiss Webster mouse since there is a significant deficit in the density of µ-receptors. Specifically, it was anticipated that chronic naltrexone treatment of CXBK mice might induce synthesis of µ-opioid receptors and an increase in µ-receptor mRNA in order to produce upregulation. However, no such increase was observed which suggests that upregulation of µ-opioid receptors depends on similar mechanisms in both the CXBK and Swiss Webster mouse. Thus, taken together with previous findings (6-8, 19), the well-established increase in µ-opioid receptor density induced by chronic opioid antagonist treatment (e.g., 1,3,4) does not appear to depend upon an increase in gene transcription in vivo. These results strongly suggest that mechanisms other than de novo synthesis of receptors are responsible for in vivo opioid antagonist-induced upregulation. While the mechanism that mediates the increase in binding sites has not been identified, several possibilities have been suggested. Among the potential mechanisms that might account for opioid antagonist-induced receptor upregulation in vivo are blockade of receptor cycling (15), decreases in receptor degradation (14) and activation of cryptic receptors (11-13, 27,34). Interestingly, upregulation of δ receptors in cell culture studies may involve different mechanisms than those that mediate in vivo upregulation, since the opioid antagonist naloxone has been reported to increase δ-opioid receptor mRNA in NG-108 cells (35).

Chronic opioid antagonist treatment produced similar supersensitivity to the analgesic effects of morphine in CXBK and Swiss Webster mice. This result is consistent with previous results showing that the increase in morphine's analgesic potency in vivo is roughly similar to the increase
in \( \mu \)-opioid receptor density (1,2,4). This also agrees with recent formulations of receptor theory (18) which predict that a 2-fold increase in receptor density will be accompanied by a similar increase in agonist potency. Thus, the starting density of \( \mu \)-opioid receptors did not impact on the magnitude of supersensitivity in the two strains of mice. However, it is notable that the ratio of basal receptor density in Swiss Webster and CXBK mice did not predict the relative potency of morphine. Specifically, \( \mu \)-opioid receptor number is \( \approx \)2-fold greater in the Swiss Webster mouse compared to the CXBK mouse, but morphine potency is \( \approx \)6-fold greater. Although our binding studies were conducted in whole brain, it is unlikely that the reduced agonist potency in the CXBK can be explained by a more pronounced deficit in \( \mu \)-receptors in select brain regions. Previous autoradiographic studies have shown that the density of \( \mu \)-receptors in the progenitor C57 strain is at most 2-fold more than that of CXBK in select brain regions that might serve the analgesic effects of opioids (17). Therefore, it is likely that other mechanisms in addition to the deficit in receptor density account for the decreased potency of morphine in the CXBK mouse. To begin to address the possible factors that might more fully explain morphine’s potency differences in the two strains, we examined the abundance of a \( G_{i} \)-protein subunit (e.g., \( G_{i\alpha 2} \) protein). We chose this particular subunit since the \( G_{i} \) protein is important in mediating opioid effects, and the \( G_{i\alpha 2} \) protein subunit has been demonstrated to play a role in morphine analgesia \textit{in vivo} (23-25, 36). We considered the possibility that a deficit in \( G_{i\alpha 2} \) protein levels might contribute to the reduced potency of morphine in CXBK mice. However, there were no differences in basal \( G_{i\alpha 2} \) and there was no effect of naltraxone treatment on whole brain \( G_{i\alpha 2} \) protein levels. These findings indicate that a deficit in \( G_{i\alpha 2} \) protein levels does not account for the decreased sensitivity to opioids in CXBK, and that opioid antagonist-induced supersensitivity may not depend upon alterations in \( G \)-proteins on whole brain (37). However, the possibility remains of differential changes in select brain areas for \( G \)-protein abundance that might account for reduced sensitivity in CXBK mice.

Previous studies demonstrated that pharmacologically induced decreases and increases in \( \mu \)-opioid receptor density do not affect the magnitude of tolerance to morphine (38,39). Therefore, taken together with the present results, receptor density does not affect the magnitude of dynamic changes in the opioid system including tolerance, supersensitivity and receptor upregulation. These results suggest that the regulatory processes involved in these changes in the opioid system do not depend on a specific level of receptor density, even though it is clear that these phenomena require receptor activation (e.g., tolerance) and occupancy (e.g., upregulation and supersensitivity). It remains to be determined if basal receptor density impacts on receptor downregulation and opioid agonist-induced reductions in \( \mu \)-opioid receptor mRNA (e.g., 31).

In summary, these data suggest that receptor number, while playing an important role in determining the potency of agonists, does not affect the magnitude of regulatory effects of chronic opioid antagonist treatment \textit{in vivo}. The adaptive responses to chronic opioid antagonist exposure were independent of opioid receptor density. Regardless of the mechanisms that account for the differences in density of \( \mu \)-opioid receptors, the processes required for receptor upregulation are intact in CXBK mice. Overall, basal receptor density does not appear to be a consideration in initiating the dynamic events that ultimately regulate receptor density and the functional consequences of receptor regulation \textit{in vivo}.

Acknowledgments

The authors thank Dr. C.J. Evans and D. Keith for providing the mMOR clone. Our thanks to Dr. M.T. Turnock for her encouragement and support. This work was supported in part by the National Institute on Drug Abuse (DA 04185). This study represents a portion of a thesis by A.D. presented in partial fulfillment of the requirement for the Ph.D. degree in Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, St. John’s University. Parts of these data
were presented at the 1997 annual meetings of the American Society for Pharmacology and Experimental Therapeutics, San Diego, CA.

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