Chapter 2
Upregulation of Opioid Receptors

Ellen M. Unterwald and Richard D. Howells

Abstract It is well established that chronic exposure to opioid receptor antagonists can result in opioid receptor upregulation. The phenomenon of antagonist-induced receptor upregulation is not unique to the opioid system but is common to many receptor systems including adenergic, cholinergic, serotinergic, and dopaminergic receptors. Chronic administration of naloxone or naltrexone reliably produces increases in binding to opioid receptors both in vivo and in vitro. This receptor upregulation is associated with functional supersensitivity to subsequent agonist administration. Thus, the analgesic potency of morphine is increased following prior exposure to opioid receptor antagonists. The three opioid receptor types show different degrees of upregulation in response to in vivo antagonist administration, with μ opioid receptors showing the largest increases in binding in response to any given dose of naloxone or naltrexone, followed by more modest increases in δ and κ receptors. Antagonist-induced receptor upregulation appears to vary between brain regions, and the reason for this is not clear. Although the first demonstration of antagonist-induced opioid receptor upregulation occurred more than 30 years ago, the mechanisms mediating this effect remained elusive for much of this time. Recent data have provided new insights into potential molecular mechanisms of opioid receptor upregulation. Data are presented that support the hypothesis that naloxone and naltrexone are acting as pharmacological chaperones, stabilizing intracellular receptor protein molecules and facilitating their trafficking and insertion into the cell membrane. Finally, heterologous opioid receptor upregulation occurs in response to repeated exposure to cocaine and ethanol, and the resulting opioid receptor regulation may play an important role in craving and reinforcement induced by these agents. Given the multiple potential clinical uses of opioid receptor antagonists described in other chapters of this volume, opioid receptor upregulation and the accompanying functional supersensitivity that results from antagonist exposure needs to be further explored in the clinical setting.

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R. Dean et al. (eds.), *Opiate Receptors and Antagonists*,

Keywords: Upregulation; Opioid receptors; Opioid antagonist; Naltrexone; Naloxone

2.1 Introduction

Opioid receptor upregulation following exposure to opioid receptor antagonists is one of the most well-documented phenomena in the field. It was first characterized over 30 years ago in nervous tissue of rodents. One of the earliest reports of opioid receptor upregulation following opioid receptor antagonist administration came from Loh and colleagues (1) as part of a larger study on the regulation of opioid receptor binding by morphine. Binding to opioid receptors in mouse brain was significantly increased 2 and 3 days after implantation of naloxone-containing pellets. Chronic administration of opioid receptor antagonists also produce functional opioid receptor supersensitivity, and this was first reported by Tang and Collins (2) who demonstrated that long-term administration of naloxone results in enhanced morphine-induced analgesia which is accompanied by an increase in the number of [^{3}H]-naloxone binding sites (3). Shortly thereafter, Herz and colleagues (4) found that chronic exposure of guinea pigs to naloxone for 1–2 weeks caused an increase in the sensitivity to opioids in the electrically stimulated longitudinal muscle-myenteric plexus ileum preparation. Once again, the enhanced inhibitory properties of opioid agonists were associated with elevations in the number of opioid receptors as measured by [^{3}H]-etorphine binding in both the guinea pig ileum and the brainstem.

The finding that exposure to opioid receptor antagonists in rodents can increase the number of opioid receptors and enhance the pharmacological effects of opioid receptor agonists has since been replicated in cell lines expressing opioid receptors. Despite the appreciation and reproducibility of this phenomenon, the mechanisms involved in opioid receptor upregulation remain elusive. Recently, new data have provided insights into potential molecular mechanisms involved in antagonist-induced opioid receptor upregulation. This chapter will review the pharmacological characteristics of antagonist-induced opioid receptor upregulation, the accompanying functional supersensitivity, and potential mechanisms involved. In addition, upregulation of opioid receptors following administration of nonopioid drugs will also be discussed.

2.2 Opioid Receptor Upregulation Following Opioid Receptor Antagonist Administration

2.2.1 In Vivo Studies

The initial reports of naloxone-induced opioid receptor supersensitivity and upregulation were followed by more detailed characterization of this phenomenon. Antagonist-induced opioid receptor upregulation occurs following chronic administration of either naloxone or naltrexone, and the antagonists are most often administered to rodents by implanting

drug-containing pellets or minipumps into the subcutaneous (sc) space. It is interesting to note that antagonist-induced upregulation of opioid receptor binding sites is observed consistently following continuous sc infusion of naloxone or naltrexone for 7 days, but not after intermittent sc injection (every 24 h for 7 days) of the same daily dose (5). Another group, however, reported that intraperitoneal (ip) injection of rats with 10 mg/kg of naltrexone for 15 days results in a significant increase in [^{3}H]-[D-Ala^2-MePhe^4-Gly(ol)^5] enkephalin (DAMGO) binding to μ receptors in the striatum (6).

Radioligand binding has been the most commonly used technique to measure opioid receptor upregulation. Increases in binding of nonselective opioid receptor ligands such as [^{3}H]-etorphine (7, 8) and [^{3}H]-naloxone (8), as well as some semi-selective opioid receptor ligands like [^{3}H]-dihydromorphine (μ ligand) (8), [^{3}H]-[D-Ala^2-D-Leu^5]enkephalin (DADLE, δ ligand) (8, 9), and [^{3}H]-ethylketocyclazocine (κ ligand) (7) occur following continuous exposure to naloxone or naltrexone. In all cases, increases in receptor number (B_{max}) rather than increases in receptor affinity (K_d) are apparent (7, 8, 10–14). Antagonist-induced receptor upregulation is stereospecific, as it is produced by the (−), but not the inactive (+) isomer of naloxone, indicating that the effect of naloxone is mediated by a specific interaction with opioid receptors (15). In addition, the sensitivity of agonist binding to inhibition by guanyl nucleotides (GTP) is increased significantly following chronic naltrexone administration suggesting augmented receptor coupling to heterotrimeric guanine nucleotide binding proteins (G-proteins) (8), although this has not been replicated in all studies (16). Zukin and colleagues reported that opioid binding reaches a maximum 8 days after naltrexone pellet implantation and is maintained at that level with continued exposure for up to 4 weeks (8). However, Giordano et al. (17) found that opioid receptor binding continues to increase for up to 60 days following naltrexone pellet implantation. Following withdrawal from chronic naltrexone, elevated opioid receptor levels return to control levels after 6 days (7).

With the advent of more selective opioid radioligands came better characterization of the regulation of the three individual opioid receptor types. Results using highly selective radioligands indicate that μ opioid receptors are most affected by naloxone or naltrexone administration, followed by δ opioid receptors (17–21). Naltrexone treatment increases the density of μ opioid receptors as measured by the μ receptor-selective ligand, [^{3}H]-DAMGO, by 80–100% in whole brain minus cerebellum, without altering receptor affinity (14, 16, 17). This is illustrated in Fig. 2.1 which shows a Scatchard plot analysis of the binding of [^{3}H]-DAMGO to membranes prepared from whole brain minus cerebellum from rats treated with saline or naltrexone for 7 days (Unterwald, unpublished data). In comparison to an 81% increase in μ receptors, binding to δ receptors in the same tissue sample is increased by 31% following naltrexone (17). Kappa opioid receptors are more resistant to regulation during naloxone or naltrexone administration (10, 19, 20). Thus, μ receptors are upregulated in response to lower doses of naloxone or naltrexone than are δ and κ opioid receptors (18, 22), and the degree of upregulation to any given dose of antagonist is greatest for μ and lowest for κ opioid receptors (18–20, 22). This may be due to differences in the molecular mechanisms involved in the regulation of the three types of opioid receptors (see Sect. 4) or rather due to the relative affinity of naloxone and naltrexone

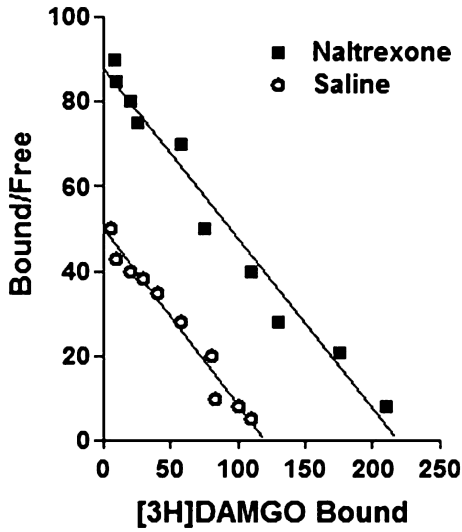

Fig. 2.1 Scatchard analysis of the binding of [^{3}H]-[D-Ala^2-MePhe^4-Gly(ol)^5] enkephalin (*DAMGO*) to mu receptors in whole brain (*minus cerebellum*) of rats exposed to saline (*open circles*) or naltrexone (8 mg/kg/day; *closed squares*) by osmotic minipumps for 7 days. Results demonstrate an 81% increase in B_{max} (120 vs 218 fmol/mg protein) following naltrexone administration. Methods are similar to those previously published (14)

for the three opioid receptors. Naloxone and naltrexone have higher affinity for μ opioid receptors than the other two opioid receptors, although their affinity at κ sites is generally reported to be greater than that for δ receptors (23, 24).

Regional analysis of naltrexone-induced opioid receptor upregulation has been performed using quantitative receptor autoradiography. Mu opioid receptors show widespread upregulation in animals exposed chronically to naltrexone or naloxone (18, 20, 25, 26). Although μ receptor upregulation occurs in most brain regions, reports are contradictory as to which areas show the greatest increase in receptor number. For example, Tempel et al. (25) report that the greatest increases in μ opioid receptor number following naltrexone administration to rats were found in brain areas associated with the A9/A10 dopamine pathway such as the nucleus accumbens, lateral septum, the patches of the striatum, amygdala, substantia nigra pars compacta, and ventral tegmental area, as well as certain nuclei in the hypothalamus and thalamus, Layer I of the neocortex and the central gray. Mu receptors were found to be elevated two- to threefold in these brain regions (25). These results are in partial agreement with those of others, with the hypothalamus, central gray, and ventral tegmental area consistently showing large increases in μ receptor binding (18, 26). However, the absolute rank order of μ receptor upregulation varies between these three papers (18, 25, 26). Chronic naltrexone exposure in the mouse results in increases in binding of the selective μ opioid receptor agonist [^{3}H]-DAMGO throughout the brain with the largest increases found in somatosensory and visual areas of the cortex. Following the cortex, the greatest increases in μ receptor binding occur in the mouse olfactory tubercle, globus pallidus, ventral pallidum, hippocampus, and hypothalamus (20).

In comparison to μ opioid receptors, upregulation of δ receptors occurs in fewer brain regions and is smaller in magnitude. In the rat, chronic naloxone produces the

largest upregulation of δ opioid receptors as measured by binding of [^{3}H]-DADLE in the amygdala, striatum, claustrum, and frontal cortex (18). In the mouse brain, upregulation of δ opioid receptor as measured by [^{3}H]-deltorphin-1 binding is widespread with the largest increases noted in the lateral septum, superior colliculus, and pontine nucleus (20). In contrast, consistent upregulation of κ opioid receptors as measured by [^{3}H]-CI-977 binding is found only in cortical brain regions of the mouse and the magnitude of this response is lower than that for μ or δ receptors (20). In the rat, κ receptors as labeled by [^{3}H]-bremazocine under conditions in which binding to μ and δ receptors is suppressed were found to be upregulated in the spinal cord, hippocampus, central gray, and frontal cortex following chronic administration of a high dose of naloxone but not lower doses (18). Comparison of the effects of chronic naltrexone on μ, δ, and κ opioid receptor binding in mouse brain is shown in Fig. 2.2. In their study, quantitative receptor autoradiography was carried out using selective radioligands for the three opioid receptors on adjacent tissue sections from the same mice. Results demonstrate once again that μ receptor upregulation is most robust and widespread and κ receptors are more resistant to upregulation.

Antagonist-induced μ receptor upregulation has also been measured using immunohistochemistry by Unterwald et al. (26). Adjacent brain sections from rats exposed continuously for 7 days to naltrexone were processed for measurement of μ opioid receptors by immunohistochemistry and by receptor autoradiography with [^{3}H]-DAMGO. In agreement with other autoradiography studies (18, 25), increased binding to μ opioid receptors was widespread and occurred in the central gray, hypothalamus, interpeduncular nucleus, ventral tegmental area, amygdala, thalamus, hippocampus, and globus pallidus. However, significant increases in μ receptor

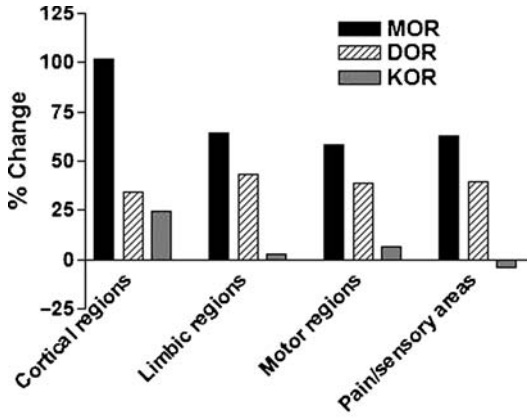

Fig. 2.2 Changes in μ (MOR), δ (DOR), and κ (KOR) opioid receptor binding in brain regions from mice exposed to naltrexone (15 mg pellet sc) for 8 days as compared with placebo pelleted controls. Receptor levels were measured by quantitative receptor autoradiography from tissue obtained 24 h after pellet removal. Results indicate that μ receptors undergo the largest upregulation in response to naltrexone treatment, whereas κ receptors were only significantly upregulated in cortical brain regions. Data adapted from Lesscher et al. (20)

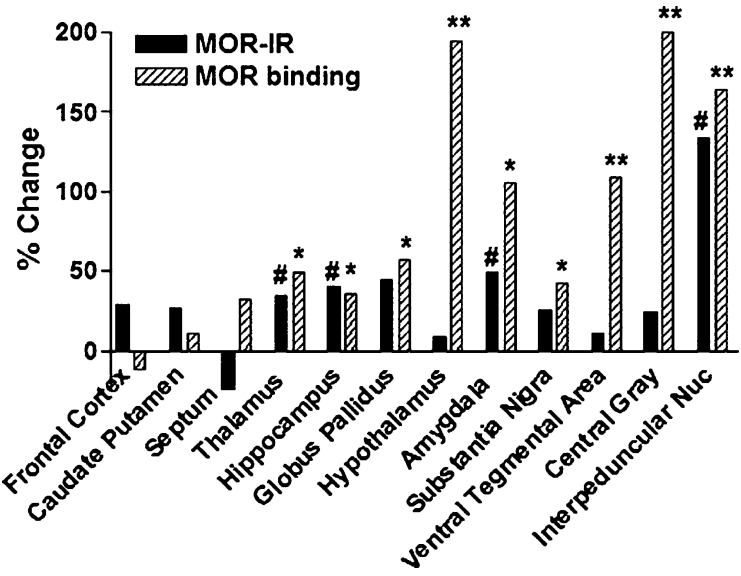

Fig. 2.3 Changes in μ receptors in various regions of rat brain following administration of naltrexone (8 mg/kg/day). Mu receptors were measured by immunoreactivity (*black bars*) or [^{3}H]-[D-Ala^2-MePhe^4-Gly(ol)^5] enkephalin (*DAMGO*) binding (*hatched bars*) on adjacent tissue sections from the same animals. Results show that increases in [^{3}H]-DAMGO binding occur in more brain regions and are generally larger in magnitude than changes in μ receptor immunoreactivity. # indicates values are significantly different from control MOR-IR levels, $p < 0.05$; * indicates values are significantly different from control MOR binding, $p < 0.05$; ** $p < 0.01$. Data adapted from Unterwald et al. (26)

immunoreactivity were limited to the interpeduncular nucleus, amygdala, hippocampus, and thalamus. Comparisons between μ receptor binding and μ receptor immunoreactivity following chronic naltrexone are illustrated in Fig. 2.3. The results indicate that chronic naltrexone exposure increases the total number of μ opioid receptors as measured by immunoreactivity only in a few brain regions, whereas μ receptor binding is increased in many brain regions. Increases in immunoreactivity are also more modest in magnitude than the increases in receptor binding, suggesting that chronic naltrexone increases the percent of active receptors without a large change in the total number of receptor molecules (26).

Opioid receptor upregulation has been well-characterized following administration of naloxone and naltrexone. In addition to these two drugs, other opioids also have been shown to produce opioid receptor upregulation in vivo. For example, Morris and Herz (27) exposed rats continuously for 7 days to bremazocine or nalorphine. Bremazocine is reported to be an agonist at κ receptors and an antagonist at μ and δ receptors (28), and nalorphine is a partial agonist at κ receptors and an antagonist at μ receptors (29, 30). Chronic administration of bremazocine results in upregulation of μ receptors, downregulation of κ receptors, and no change in δ receptors. Nalorphine produces a doubling of μ receptors and no changes in κ receptor

density (27). These data demonstrate that opioid receptor upregulation and downregulation can occur simultaneously. In addition, regulation of opioid receptor expression depends on the intrinsic activity and relative receptor selectivities of the ligand.

2.2.2 In Vitro Studies

Upregulation of opioid receptors following antagonist exposure not only occurs in vivo, but it has also been demonstrated in vitro. In neuroblastoma-glioma NG108-15 cells which endogenously express δ opioid receptors, receptor upregulation has been found although inconsistently. For example, NG108-15 cells cultured for 48 h with naloxone showed increases in opioid receptor binding in discrete cell membrane fractions (31, 32). In contrast, Law et al. (33) found no changes in opioid receptor binding following 24-h incubation with naloxone. Binding to upregulated δ opioid receptors in naloxone-treated NG108-15 cells reported by Barg et al. was not affected by guanyl nucleotides (32). Increases in [^{3}H]-DADLE and [^{3}H]-diprenorphine binding was also found by Belcheva et al. (34) following exposure of NG108-15 cells for 48 h to naltrexone or the δ receptor antagonist ICI174864. Similar to the findings in brain, receptor number in NG108-15 cells increases without changes in receptor affinity. Upregulation of opioid receptors has been documented in the human neuroblastoma cell line SH-SY5Y which expresses μ and δ receptors at a ratio of 1.4 to 1 (35). Naloxone increases both μ and δ opioid receptor densities in a dose-dependent manner in SH-SY5Y cells (35, 36). The selective μ receptor antagonist D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH2 (CTAP) increases μ and decreases δ opioid receptor densities in these cells, whereas ICI174864 upregulates δ and to a lesser extent μ receptors (35). It has been reported that CTAP at high concentrations has intrinsic agonist activity at the δ receptor in the mouse vas deferens (37) and this might explain the downregulation of δ opioid receptors following CTAP. CTAP-induced downregulation of δ receptors is blocked by ICI174864 (35).

Antagonist-induced opioid receptor upregulation has been studied in transfected cell lines. Human embryonic kidney 293 (HEK293) cells stably expressing the murine μ opioid receptor showed significant increases in surface μ receptors using flow cytometic analysis (38). One-h treatment with naloxone produces a 16% increase in surface receptor staining and an 18-h exposure produces a 39% increase in staining. Buprenorphine, a weak partial μ agonist, also causes an upregulation of μ receptors in these cells, although the magnitude is lower than that produced by equivalent concentrations of naloxone. Addition of pertussis toxin augments the increase in surface receptor staining caused by 18 h of naloxone from 39% to 70%. Thus, pertussis toxin which inhibits the activation of Gi/Go proteins by the receptor (39) modulates the ability of the antagonist to regulate surface receptors (38). In Chinese hamster ovary (CHO) cells stably expressing the rat μ opioid receptor, naloxone increases receptor binding in whole-cell preparations in a concentration- and time-dependent manner, reaching a plateau of about 45% above control levels at

72 h (40). These data indicate that opioid receptors expressed in cell lines undergo upregulation in response to exposure to opioid receptor antagonists with similar pharmacological properties as those in the whole animal.

2.3 Functional Supersensitivity

Chronic exposure to opioid receptor antagonists not only produces an increase in the number of opioid receptor binding sites but also increases the subsequent response to opioid receptor agonists. This was first reported by Tang and Collins (2) who found that long-term treatment with naloxone results in enhanced morphine-induced analgesia and that the enhanced analgesic response is associated with an increase in receptor binding (3). Likewise, Herz and colleagues (4) found that chronic exposure of guinea pigs to naloxone for 1–2 weeks increases the sensitivity to opioids in the electrically stimulated longitudinal muscle-myenteric plexus ileum preparation. The enhanced inhibitory properties of opioid agonists occur together with elevations in the number of opioid receptors as measured by [^{3}H]-etorphine binding in both the guinea pig ileum and the brainstem.

These initial observations were followed by many other reports of functional supersensitivity to opioid receptor agonists after chronic antagonist administration. Long-term exposure to naloxone or naltrexone results in supersensitivity to morphine analgesia as demonstrated by a leftward shift in the morphine analgesic dose-response curve (10, 12, 22, 41). In agreement with the receptor binding data (7), sensitivity to morphine on analgesic tests returns to baseline levels 6 days after cessation of naltrexone administration (10, 22). Supersensitivity to other opioid analgesics also occurs including methadone, etorphine, fentanyl, meperidine, and oxycodone (13). The degree of receptor upregulation coincides with changes in agonist potency. Thus, μ and δ receptor bindings are increased by 81% and 31%, respectively, in mouse whole brain following 8 days of naltrexone administration. Consistent with the binding changes, the potency of morphine administered intracerebroventricularly (icv) to produce analgesia is increased by threefold, whereas the potency of DADLE is increased by 1.7-fold (17). In contrast to the functional supersensitivity to morphine following systemic antagonist administration, it has been shown that chronic spinal infusion of naloxone or naltrexone fails to influence the antinociceptive effect of subsequent intrathecal morphine administration on the hot plate test in rats (42).

Other effects of opioid agonists are also exaggerated following antagonist administration. Six injections of naloxone over 3 days are sufficient to produce an increase in locomotor response to subsequent morphine administration in C57Bl6 mice (43). Likewise, the hyperthermic response to acute morphine administration is enhanced following chronic naltrexone (12). Neurons in the locus coeruleus of chronic naltrexone-treated rats exhibit enhanced inhibitory responses to morphine (44). Augmented morphine withdrawal signs are seen when the morphine treatment is preceded by chronic naloxone (45). The lethality of morphine

is increased 2.5-fold following chronic naltrexone treatment in the mouse (46). The ability of μ opioid receptors to activate G-proteins and subsequently inhibit cAMP production is also enhanced. Activation of G-proteins by μ opioid receptor agonists as measured by [^{35}S]GTPγS binding is augmented in mouse spinal cord following 7 days of naloxone injections (47), indicating enhanced receptor G-protein coupling. Chronic naltrexone also augments the efficacy of opioid receptor agonists to inhibit adenylyl cyclase activity (16). Taken together, these results indicate that chronic exposure to opioid receptor antagonists leads to an increase in opioid receptor number and an increase in functional opioid receptors.

2.4 Studies of the Molecular Mechanisms Involved in Antagonist-Induced Opioid Receptor Upregulation

Chronic administration of morphine or other opioid drugs for pain relief results in tolerance to the analgesic effects, and physical dependence which becomes evident in the characteristic opioid withdrawal syndrome upon abrupt cessation of drug use (48). In contrast, chronic blockade of opioid receptors with opioid antagonists such as naloxone or naltrexone does not result in physical dependence, and was shown nearly 30 years ago to be associated with supersensitivity to the analgesic actions of morphine using the tail shock-vocalization test in rats (2). As discussed above, continuous infusion of naloxone for 4 weeks in rats causes a 40% increase in the number of [^{3}H]-naloxone binding sites with no change in affinity, indicating that the enhanced analgesic effects of morphine are correlated with an increase in the number of opioid receptor binding sites (3, 4).

Opioid receptor activation regulates the activity of adenylyl cyclase, potassium channels, calcium channels, and mitogen-activated protein kinase in a pertussis toxin-sensitive manner (via G_i/G_o heterotrimeric guanine nucleotide binding proteins), therefore it was of obvious interest to determine whether antagonist-induced opioid receptor upregulation was also inhibited by the toxin. Yoburn and colleagues (49) treated mice chronically for 8 days with naltrexone with and without pertussis toxin, and the increase in [^{3}H]-DADLE binding to δ receptors and [^{3}H]-DAMGO to μ receptors was not altered in animals pretreated with pertussis toxin. Supersensitivity to morphine analgesia following naltrexone treatment, however, was blocked by pertussis toxin pretreatment, suggesting that morphine analgesia requires opioid receptor coupling to G_i/G_o heterotrimeric guanine nucleotide binding proteins while the antagonist-induced upregulation of opioid binding sites does not.

Following the molecular cloning of μ, δ, and κ opioid receptors, it became feasible to determine whether the increase in opioid binding sites following chronic antagonist treatment is associated with an increase in the steady-state level of opioid receptor mRNA. Unterwald et al. (14) found that 7-day infusion of naltrexone significantly upregulates μ opioid receptor binding rat brain; however, μ opioid receptor mRNA levels are not significantly altered in any brain region. Similar results regarding the lack of an effect of antagonist treatment on μ opioid receptor

mRNA levels were reported subsequently by others (6). Jenab and Inturrisi (50) reported that treatment of NG108-15 cells (that express the δ opioid receptor endogenously) with 1 μM naloxone for 24 or 48 h causes a twofold increase in the level of δ opioid receptor mRNA as measured by solution hybridization or Northern blot analysis. Work from our laboratory, however, has not confirmed this result in NG108-15 cells using similar protocols (Wannemacher et al., submitted). Chronic treatment of mice with naltrexone for 7 days results in an eightfold increase in the antinociceptive potency of [D-Ala^2]deltorphin II as measured by the tail-flick test but does not change the levels of δ opioid receptor mRNA in any brain area tested (51). Thus, it appears that posttranscriptional mechanisms are involved in antagonist-induced opioid receptor upregulation, and that changes in the steady-state level of opioid receptor mRNAs do not occur in response to chronic antagonist treatment, either as a result of increased transcription or decreased degradation of the receptor transcripts.

A large number of human diseases, including cystic fibrosis, emphysema, and several neurological disorders, are due to inefficient protein folding resulting from amino acid substitutions, deletions, or insertions that arise from genetic mutations (52, 53). It has been observed that glycerol and other "chemical chaperones" can facilitate proper folding of the mutant cystic fibrosis transmembrane conductance regulator (54), mutant α1-antitrypsin (55), temperature-sensitive folding mutants of p53 (56), prion proteins (57), and defective aquaporin-2 associated with nephrogenic diabetes insipidus (58). It has also been found that a competitive inhibitor of lysosomal α-galactosidase A is able to accelerate the transport and maturation of the mutant form of this protein associated with Fabry disease (59).

There are also several examples in which mutant forms of G protein-coupled receptors are responsible for human diseases. Mutant rhodopsins cause retinitis pigmentosa (60), mutated forms of the luteinizing hormone receptor cause several endocrine disorders in males and females (61), mutant gonadotropin-releasing hormone receptors cause hypogonadotropic hypogonadism (62), and a large number of mutations in the vasopressin V2 receptor are responsible for X-linked nephrogenic diabetes insipidus (63). Many of these mutations cause improper folding during synthesis of the G protein-coupled receptor, and expression levels are reduced as a result of proteolysis by the endoplasmic reticulum quality control system (64). In this system, misfolded membrane proteins are deglycosylated, ubiquitinated, and then degraded by the 26S proteasome.

In an elegant study by Morello et al. (65), it was reported that selective nonpeptidic V2 vasopressin receptor antagonists increase cell-surface expression and can rescue the function of several mutant forms of the receptor that cause human X-linked nephrogenic diabetes insipidus by promoting proper folding and maturation. A cell impermeant V2 receptor antagonist is inactive in this regard, and does not block the rescue activity of the cell-permeable antagonist. The authors suggest that the active antagonists acted intracellularly as "pharmacological chaperones," by binding to and stabilizing the newly synthesized mutant receptors, thereby promoting proper folding, maturation, exit from the endoplasmic reticulum, and trafficking to the cell surface. Interestingly, the cell-permeable, active antagonist

does not increase the cell surface expression of the wild-type V2 vasopressin receptor. More recently, it has been found that pharmacological chaperones can rescue mutant forms of gonadotropin-releasing hormone receptors causing hypogonadotropic hypogonadism in humans (62).

The rhopopsin family of G protein-coupled receptors, which the opioid receptors are members of, has an invariant (D/E)RY amino acid sequence located on the cytoplasmic surface of the third transmembrane domain. Substitution of the D/E in this motif with other amino acids results in constitutively active mutants of rhodopsin (66), the α_{1B}- (67) and β_2-adrenergic receptors (68), and the μ opioid receptor (69). Substitution of the aspartic acid in the DRY sequence of the μ opioid receptor with glutamine, histidine, methionine, or tyrosine completely abolishes [^{3}H]diprenorphine binding and reduces receptor expression to undetectable levels in transfected HEK293 and CHO cells; however, inclusion of naloxone in the cell culture media for 96 h greatly enhances the mutant receptor binding activity and expression levels (69). In that study, it was reported that naloxone has little or no effect on wild-type μ receptor binding or immunoreactivity in transfected HEK293 or CHO cells. The authors concluded that naloxone is acting as an inverse agonist to block the agonist-independent constitutive downregulation of the mutant receptor, and naloxone also decreases the rate of denaturation of the mutant receptor binding site (69). Liu-Chen and colleagues subsequently reported that naloxone increased [^{3}H]-diprenorphine binding and protein expression of the D164Q μ opioid receptor without affecting its mRNA level (40). Coexpression of dominant negative forms of GRK2, arrestin, dynamin, rab5A, and rab7 partially prevents the decline in [^{3}H]-diprenorphine binding following removal of naloxone from the culture media of CHO cells transfected with the mutant, and protease inhibitors also partially block the loss of [^{3}H]-diprenorphine binding after naloxone removal. It was concluded that naloxone upregulated the mutant D164Q μ receptor by stabilizing its binding site and inhibiting constitutive internalization and downregulation (40). Mutation of the analogous amino acid, D148A, in the vasopressin V_{1a} receptor also results in a misfolded but nonfunctional intracellular receptor, and the nonpeptide antagonist, SR49059, dramatically increases the cell surface expression and functionality of the mutant receptor (70). The rescue does not involve de novo receptor synthesis or preventing constitutive activity or internalization.

It has been reported that a large fraction (30%) of newly synthesized proteins are degraded by the proteasome as a result of targeting by the endoplasmic reticulum quality control system (71). Thus, not only are mutated, misfolded proteins recognized and degraded by the endoplasmic reticulum quality control, but other "normal" proteins that are intrinsically difficult and slow to fold properly during and shortly after synthesis are also subject to quality control. Studies of δ opioid receptor expression in transfected HEK293 cells reveal that as little as 40% of the newly synthesized receptors are exported out of the endoplasmic reticulum (72), and receptors retained in the endoplasmic reticulum are subsequently ubiquitinated and targeted to the proteasome (73). Chaturvedi et al. (74) demonstrated that the proteasome is also involved in both basal turnover and agonist-induced downregulation of μ and δ opioid receptors expressed in transfected HEK293 cells, and

δ opioid receptors expressed endogenously in NG108-15 cells. Using pulse-chase analysis to follow newly synthesized receptors, Bouvier and colleagues found that cell-permeable opioid receptor agonists and antagonists can promote maturation and exportation of δ opioid receptors expressed in HEK293 cells from the endoplasmic reticulum through the Golgi network to the plasma membrane (75). The cell-impermeable peptide, leu-enkephalin, does not increase the efficiency of receptor maturation, and does not inhibit the action of naltrexone when administered together, suggesting that naltrexone acts intracellularly. Further evidence for an intracellular site of action for opioid receptor agonists and antagonists was provided in experiments using brefeldin A, which blocks intracellular trafficking of proteins from the Golgi apparatus to the plasma membrane (76). When transfected HEK293 cells are pulse-chased and incubated with opioid alkaloid agonists and antagonists in the presence of brefeldin A, the cell-permeable ligands stimulate the accumulation of a labeled receptor intermediate in an intracellular compartment. Bouvier and colleagues also found that the D95A substitution in the second transmembrane domain of the δ receptor results in significant retention of the receptor precursor in the endoplasmic reticulum, and that cell-permeable antagonists increase maturation and exit of the receptor from the endoplasmic reticulum and increase the cell surface expression of the mutant (75).

Chaipatikul et al. (77) report that a variety of hydrophobic antagonists and agonists can increase the cell surface expression of mutant μ opioid receptors. Deletion of the RLSKV sequence in the third intracellular loop or the KRCFR sequence in the proximal C-terminus of the rat μ opioid receptor leads to low levels of expression in transfected HEK293 cells. Naloxone causes a time- and concentration-dependent three- to fourfold increase in cell-surface expression and a fivefold increase in [^{3}H]-diprenorphine binding to the mutant receptors but has no effect on cell surface expression or binding to the wild-type μ receptor. In this study (+)-naloxone, the inactive isomer, and naloxone methiodide, the positively charged quaternary analog, lack the ability to increase cell surface expression of the mutant and wild-type μ opioid receptors (77). CTOP, the selective μ receptor peptide antagonist, has no effect on surface expression of the wild-type or mutant receptors. DAMGO, the selective μ receptor peptide agonist, morphine, and etorphine decrease the cell surface expression of the wild-type receptor, and morphine and etorphine, but not DAMGO, increase the surface expression of the μ mutant receptors. The ability of morphine to decrease the cell surface expression of the wild-type μ receptor was unexpected, since it has often been observed that morphine, unlike most other μ receptor agonists, does not stimulate internalization of the μ opioid receptor (78). With the use of confocal immunofluorescence microscopy, it was shown that the mutant μ receptors colocalize with the endoplasmic reticulum chaperone protein, calnexin, while in the presence of naloxone or etorphine, the mutated receptors are located predominantly on the cell surface (77). Brefeldin A completely blocks the action of naloxone to increase the cell surface expression of the mutant receptors, providing further evidence that the antagonist is affecting intracellular trafficking of the mutant receptors. Agonist activation of the mutant μ receptor with the RLSKV deletion does not inhibit adenylyl cyclase activity efficiently even after transfected

cells are treated with naloxone; however, naloxone treatment increases the maximal inhibition of the KRCFR-deleted mutant significantly. Since the KRCFR-deleted mutant is capable of signaling to an effector upon agonist stimulation, it is not clear why that mutant receptor did not downregulate following 48-h treatment with potent agonists like etorphine, levorphanol, and methadone (77). Deletion of the RLSKV or KRCFR sequences from the μ opioid receptor do not cause the mutant receptors to become constitutively active, hence the mechanism for the mutant receptor upregulation has to differ from that proposed by Liu-Chen and colleagues (40), and Chaipatikul et al. (77) proposed that the hydrophobic μ ligands were acting like chaperones to promote intracellular trafficking of the mutant receptors.

Howells and colleagues studied antagonist-induced upregulation of the mouse δ opioid receptor using transfected HEK293 cells stably expressing FLAG-tagged receptors (79, 80). Following 24-h incubation with either naltrexone or naloxone, the B_{max} of the δ-expressing cells increases twofold as assessed by [^{3}H]-diprenorphine binding, with no apparent change in affinity. Western blot analysis following antagonist treatment revealed that there is no increase in the main immunoreactive δ receptor species migrating at 60 kDa, and the level of a minor receptor form migrating at 40 kDa is decreased. The 60 kDa δ receptor species contains complex N-glycans, and is most likely responsible for high-affinity ligand binding. Cell surface biotinylation assays show that the 40 kDa δ receptor band is located entirely intracellularly. Naltrexone does not have any affect on δ receptor mRNA as assessed by quantitative real-time PCR, or δ receptor translational efficiency as determined by [^{35}S]-methionine and cysteine incorporation. From these observations, we propose that opioid receptor antagonists facilitate the folding of a low-affinity desensitized pool of δ receptors resulting in increased binding without an increase in total immunoreactive receptor protein.

We also studied ligand-induced regulation of the FLAG-tagged rat κ opioid receptor in transfected HEK293 cells (79, 81). Receptor levels were determined following agonist or antagonist treatment by saturation analysis using [^{3}H]-diprenorphine or by Western blotting with the anti-FLAG M1 monoclonal antibody for detection. Treatment of cells expressing the κ opioid receptor with naltrexone produces a time-dependent increase in the κ opioid receptor B_{max} with no apparent change in K_d, with a maximal threefold increase at 8 h. Following exposure for 24 h with 1 μM dynorphin A 1–13, a selective κ receptor peptide agonist, or 1 μM U69593, a selective κ receptor arylacetamide agonist, κ opioid receptor levels were unaffected when determined by binding assays or Western blotting. Thus, the rat κ opioid receptor is unusual in that long-term agonist treatment does not cause receptor downregulation, as previously reported (82). To our surprise, incubation of cells with etorphine or cyclazocine, both κ receptor alkaloid agonists, *increased* κ opioid receptor immunoreactivity (81). Similar results were obtained following incubation with the antagonists, naltrexone, and naloxone. Western blot analysis revealed a time-dependent increase in a 52 kDa κ opioid receptor immunoreactive species that was similar in magnitude to the increase as assessed by ligand binding. In addition, a 42 kDa κ opioid receptor immunoreactive species was decreased in a time-dependent manner following treatment with these ligands. Both κ opioid recep-

tor bands accumulate in the presence of MG132, a proteasome inhibitor, indicating that the proteasome is involved in turnover of the κ receptor. We found that the 52 kDa band bound tightly to wheat germ agglutinin-agarose, whereas the smaller species did not, indicating the larger species contains terminal N-acetylglucosamine residues. Enzymatic digestion with PNGase F and endoglycosidase H indicated that the 52 kDa κ opioid receptor species contains complex N-glycans while the 42 kDa κ opioid receptor species contains N-glycans of the high mannose type, suggesting the 42 kDa κ opioid receptor species is a precursor to the 52 kDa species, and pulse-chase analysis confirmed this (81). Naltrexone did not have any effect on κ opioid receptor mRNA as assessed by quantitative real-time PCR, or κ opioid receptor translational efficiency as determined by [^{35}S]-methionine and cysteine incorporation. Naltrexone treatment does, however, more than double the rate of conversion of the 42 kDa precursor to the mature 52 kDa species, as determined by pulse-chase analysis. Cotreatment of κ opioid receptor cells with naltrexone and brefeldin A, an inhibitor of the secretory pathway, caused the stabilization of an intracellular 46 kDa κ opioid receptor intermediate. Taken together, these results suggest that naltrexone and other select ligands upregulate κ opioid receptor by entering the cell and enhancing the rate of receptor maturation through the secretory pathway and by protecting the receptor from degradation by the proteasome. Cellular uptake studies confirm that [^{3}H]-naloxone and [^{3}H]-U69593 are cell permeable (81). Dynorphin A(1–13) cannot upregulate the κ receptor, presumably because it cannot enter the cell due to its peptidic nature; however, cell permeability is not sufficient for ligand-induced upregulation since U69593 enters the cell but does not stimulate receptor upregulation. To further confound the situation, we found that incubation of κ opioid receptor cells with naloxone methiodide, a quaternary analog of naloxone that is positively charged, increased the κ opioid receptor B_{max} to a similar extent as naloxone (81). Moreover, dynorphin did not block the upregulation induced by naloxone methiodide, suggesting that naloxone methiodide can actually enter the cell despite the common assumption that it does not.

Liu-Chen and colleagues recently published studies on ligand-induced regulation of the human κ opioid receptor expressed in CHO cells (83). It was reported that 4-h exposure to the peptide agonists, dynorphin A and B, downregulates the mature 55 kDa form of the human κ receptor by 70%, while several other nonpeptide agonists tested such as U50488H cause downregulation but to a lesser extent (20–30%). In contrast, the nonpeptide ligands etorphine (a full agonist), pentazocine (a partial agonist), and the antagonists, naloxone and norbinaltorphimine, cause a 15–25% increase in the 55 kDa κ receptor species. Pulse-chase experiments indicate that naloxone slightly increases the extent of conversion of a 45 kDa precursor to the 55 kDa mature form, with no apparent effect on the stability of the mature form following an 8-h chase. Following metabolic labeling in the presence of brefeldin A, naloxone increases the level of a 51 kDa intracellular human κ receptor intermediate. All nonpeptide agonists tested also increase the level of the 51 kDa species that appears in the presence of brefeldin, demonstrating that these agonists can also enter the cell and promote the maturation of the human κ opioid receptor. It was proposed that nonpeptide agonists cause less downregulation of the human

κ opioid receptor than peptide agonists due to their pharmacological chaperone activity counteracting the extent of downregulation, although it was not clear why etorphine had such low efficacy in downregulating the receptor, particularly in the presence of brefeldin A.

Long-term exposure to nicotine elicits upregulation of nicotinic acetylcholine receptors in rodent brain and in human cigarette smokers (84–86). Sallette et al. (87) recently reported that in transfected HEK293 cells expressing human α4β2 nicotinic receptors, high mannose, glycosylated subunits mature and assemble into pentamers in the endoplasmic reticulum and only pentameric receptors reach the plasma membrane following carbohydrate processing. Nicotine was found to act intracellularly to increase assembly of pentamers. Kuryatov et al. (88) expressed human nicotinic acetylcholine receptor α4 subunits or mutant α4 subunits found in autosomal-dominant nocturnal frontal lobe epilepsy in HEK cells and studied their sensitivity to activation, rate of desensitization, and ligand-induced upregulation. Upregulation was due to an increase in assembly of nicotinic receptors from pools of subunits and from a fivefold increase in the lifetime of receptors at the cell surface. Nicotine and less permeable quaternary amine cholinergic ligands act as pharmacological chaperones in the endoplasmic reticulum to facilitate the assembly of pentameric receptors. In contrast, Green and colleagues reported that the four- to sixfold increase in binding to α4β2 nicotinic receptors following nicotine exposure does not correspond to an increase in receptors at the cell surface or a change in the assembly, trafficking or turnover of receptors at the cell surface (89). They propose that nicotine slowly stabilizes the α4β2 receptor in a high-affinity state that is more easily activated and slower to desensitize.

Taken together, the opioid receptor studies indicate that the molecular mechanism involved in ligand-induced δ and κ receptor upregulation is not associated with an increase in receptor mRNA or an increase in the efficiency of mRNA translation. Antagonists apparently act as pharmacological chaperones to facilitate the folding of a low-affinity desensitized pool of δ receptors resulting in increased binding without an increase in total cellular immunoreactive protein. Further, our results suggest that naltrexone and other select κ ligands upregulate κ opioid receptors by entering the cell and enhancing the folding and rate of receptor maturation through the secretory pathway and by protecting the receptor from degradation by the proteasome, resulting in an increase in the number of κ receptor binding sites and an increase in the level of κ receptor immunoreactive protein at the cell surface, as shown in the model displayed in Fig. 2.4.

2.5 Opioid Receptor Upregulation Induced by Nonopioid Drugs

Upregulation of opioid receptor number and function following chronic opioid receptor antagonist administration is well documented, as described in the preceding sections. Other classes of drugs have also been shown to produce an upregu-

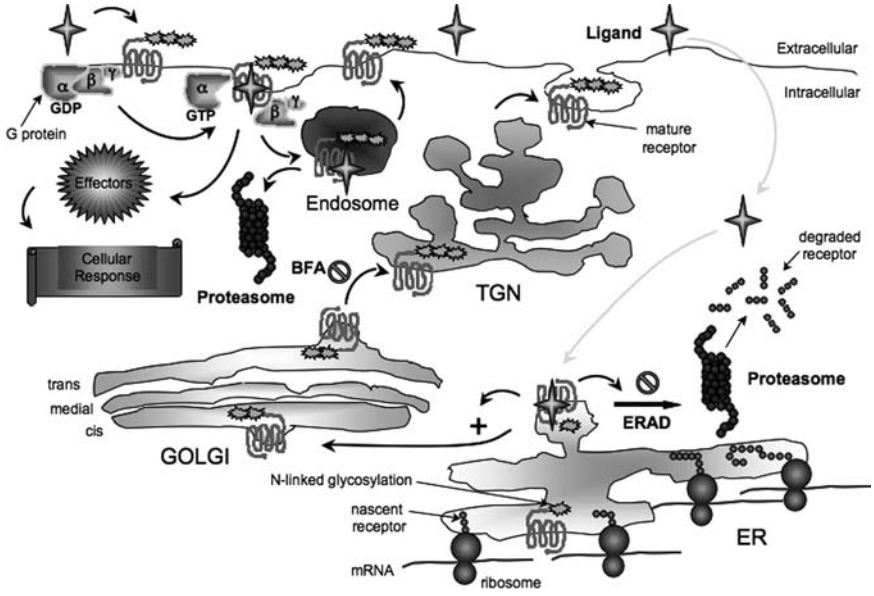

Fig. 2.4 Ligand-induced regulation of the kappa opioid receptor. The kappa opioid receptor is synthesized and partially glycosylated in the rough endoplasmic reticulum (*ER*). Proper receptor folding is monitored in the ER, and misfolded receptors are removed by the proteasome-dependent endoplasmic reticulum-associated degradation (*ERAD*) machinery. Evidence suggests that receptor upregulation is mediated by kappa ligands that enter the cell and engage incompletely processed receptor intermediates in the ER, thereby stimulating proper folding and transport to the Golgi, and limiting proteolysis by ERAD. Correctly folded receptors traverse the Golgi where they are further processed, then pass to the trans-Golgi network (*TGN*) where receptor maturation is completed, before vesicle-mediated insertion in the plasma membrane. Brefeldin A (*BFA*) inhibits transport from the Golgi apparatus to the TGN, and causes fusion of the Golgi and ER compartments. Mature receptors on the cell surface interact with kappa agonists to stimulate G-protein activation and effector regulation, leading to altered cellular responses. Activated receptors undergo endocytosis and are subsequently either recycled back to the plasma membrane or are degraded (receptor downregulation)

lation of opioid receptors, including dopaminergic agents. For example, in vivo administration of cocaine can profoundly affect the expression and function of opioid receptors. Cocaine administered to rats for 14 days results in an increase in μ and κ opioid receptors as measured by quantitative receptor autoradiography (90–92). Unlike the effects of naltrexone or naloxone, cocaine-induced opioid receptor upregulation is regionally confined, such that cocaine causes an increase in μ opioid receptors only in the basolateral amygdala, the rostral aspects of the cingulate cortex, caudate putamen, and nucleus accumbens. Kappa opioid receptors are significantly increased in the cingulate cortex, rostral caudate putamen, caudal olfactory tubercle, and ventral tegmental area. In contrast, significant regulation of δ opioid receptors does not occur following chronic cocaine administration (91). The brain regions that show the greatest regulation of opioid receptors following

cocaine administration are regions that contain major dopaminergic pathways. Since cocaine inhibits the reuptake of dopamine thereby acting as an indirect agonist at dopamine receptors, these results suggest that alterations in dopaminergic neurotransmission may play a role in the regulation of μ and κ opioid receptors. In support of this, chronic administration of the D2 dopamine receptor agonist quinpirole also produces an upregulation of μ, but not δ opioid receptors in mouse striatum, whereas chronic administration of the selective D1 receptor agonist SKF38393 is ineffective in altering opioid receptor binding (93). Conversely, administration of a D2 receptor antagonist can reduce levels of striatal μ opioid receptors (94), possibly due to an increase in striatal enkephalin (95, 96). Immunohistochemistry at the electron microscope level has demonstrated that μ opioid and D2 dopamine receptors are coexpressed in individual neurons of the striatum (97), permitting the possibility of an intracellular mechanism for μ receptor regulation by D2 receptor activation.

Cocaine-induced μ receptor upregulation is both dose-dependent and time-dependent with time course studies indicating that chronic administration of cocaine (7 or, in most cases 14 days) is needed to produce an upregulation in μ opioid receptor binding (92) and that acute cocaine is without effect (92, 98). Interestingly, however, increases in μ receptor function as measured by activation of G-proteins, is seen earlier, after only 3 days of binge-pattern cocaine administration in the striatum (99). The upregulation of μ opioid receptors following binge-pattern cocaine persists for at least 14 days after cocaine cessation (100).

The schedule of cocaine administration can influence the extent of opioid receptor regulation. Comparison of 30 mg/kg cocaine given as a single daily injection versus two 15 mg/kg injections spaced 12 h apart versus three 10 mg/kg injections given at 1-h intervals (binge-pattern) demonstrated that cocaine administered in a binge-pattern produced the greatest degree of opioid receptor upregulation (101), suggesting that frequency of administration is important to the degree of receptor regulation. It has been shown that continuous administration of cocaine delivered by subcutaneously implanted minipumps also increases μ opioid receptor binding in rat brain (102, 103). The pattern of receptor regulation across brain regions varies with the dose and method of drug delivery, although upregulation of μ opioid receptors in the nucleus accumbens appears to be a consistent finding.

In addition to occurring in rodents, opioid receptor upregulation following cocaine exposure also occurs in humans and may play a role in cocaine addiction. Binding to μ opioid receptors was measured in cocaine-dependent men and nonaddicted control subjects using positron emission tomography (PET) with the selective μ receptor ligand [^{11}C]-carfentanil (104, 105). Results from these studies show that μ opioid receptor binding is significantly increased in several brain regions of the cocaine-addicted persons when studied 1–4 days after their last use of cocaine. Binding to μ opioid receptors is increased in the caudate nucleus, thalamus, cingulate cortex, frontal cortex, and temporal cortex. Interestingly, self-reports of craving for cocaine collected at the time of the PET scan were positively correlated with μ receptor binding in the amygdala, anterior cingulate cortex, frontal cortex, and temporal cortex (104). After an additional 4 weeks of monitored drug abstinence,

μ receptor binding remained increased in most brain regions, although there was no longer a significant correlation with cocaine craving (104). Elevations in μ receptor binding in the anterior cingulate and anterior frontal cortex are still evident after 12 weeks of cocaine abstinence (105). Binding to μ opioid receptors is significantly correlated with the percentage of days of cocaine use and amount of cocaine used per day during the 2 weeks before the first scan and also with urine cocaine metabolite (benzoylecgonine) concentrations at the time of the first scan (105), suggesting a significant dose–response relationship between cocaine and μ receptor changes.

Tissue from postmortem human brains has been used to investigate the regulation of opioid receptors in cocaine-exposed individuals. In contrast to the findings from the PET studies, Hurd and Herkenham (106) report decreases in binding to μ opioid receptors in the caudate nucleus and putamen of persons who have died with positive urine toxicologies for cocaine. The disparate findings between the two studies could be due to methodological issues in measuring binding to μ receptors in living humans versus postmortem tissue. Differences may also be attributed to the differences in duration of cocaine use and amount of cocaine used, as it was found that these factors are significantly correlated with μ receptor upregulation (105). Another potential confound is that many of the postmortem samples came from persons who also tested positive for other drugs such as ethanol (106) which is known to influence the endogenous opioid system (107; 98; see discussion below). In another study using postmortem human brain tissue, binding to κ_2 receptors in the nucleus accumbens and other limbic brain regions was found to be twofold higher in fatal cocaine overdose victims than in age-matched and drug-free control subjects (108). The authors suggest that upregulation of κ opioid receptors may underlie in part the dysphoric mood and psychological distress associated with abrupt withdrawal of cocaine. Similar increases in binding to κ opioid receptors in striatum of human cocaine addicts were reported by Hurd and Herkenham (106). Taken together, these studies demonstrate that opioid receptors can be modulated by cocaine exposure and suggest a potential role of the endogenous opioid system in cocaine addiction.

Heterologous opioid receptor upregulation has also been shown to occur in response to ethanol both in animals and in cell lines. In contrast to the effects of cocaine, ethanol appears to have its greatest effects on δ opioid receptors. Early studies demonstrated that brains obtained from mice fed an ethanol-containing diet for 5 days had altered binding of [^{3}H]-DADLE, without a change in [^{3}H]-naloxone binding (109). In the mouse neuroblastoma-rat glioma hybrid cell line, NG108-15, exposure to high concentrations of ethanol (200 mM) increases opioid receptor binding after 18–24 h, whereas lower concentrations (25–50 mM) produces similar changes after 2 weeks. Opioid receptor density increases by twofold without a change in receptor affinity (110). Ethanol-induced opioid receptor upregulation is accompanied by an increase in receptor function, as shown by a 3.5-fold increase in the potency of etorphine for inhibiting phenylisopropyladenosine-stimulated cAMP accumulation (111). Subsequent studies found that δ opioid receptor mRNA transcript levels in NG108-15 cells are increased two- to threefold after exposure to

200 mM ethanol (50, 112). Delta opioid receptor mRNA levels peak at 24–48 h after ethanol exposure (50).

More recent work in rodents had yielded mixed results. Increases in binding of the selective δ receptor agonist, [^{3}H]-[D-Pen2, D-Pen5]-enkephalin (DPDPE), have been reported in rats exposed to acute ethanol. Using quantitative receptor autoradiography with [^{3}H]-DPDPE, δ opioid receptor upregulation was found in dopaminergic brain regions 1–2 h after acute ethanol administration by the oral route (2.5 g/kg) (113). In contrast, using receptor autoradiography Rosin and colleagues (98) found no changes in [^{3}H]-deltorphin-I binding to δ receptors 3 h after an acute administration of ethanol by the ip route (2 g/kg) (98). Other studies have shown that chronic exposure to ethanol, for example, given in the drinking water for 1 week to 1 month, does not alter δ receptor binding in the striatum of rat brain (114) or brain or spinal cord of the mouse (115). Changes in opioid receptor function, however, have been noted including a 1.6- to 2-fold decrease in the analgesic potency of morphine and the δ opioid receptor agonist DSLET in the mouse talk flick assay (115), as well as alterations in DADLE-inhibited adenylyl cyclase activity (116). However, others have found no changes in δ receptor-mediated G-protein activity in rats allowed to self-administer ethanol for 1 month (117). Using the alternative approach of measuring opioid receptors by immunohistochemical analysis on brain sections, Saland et al. (118) noted increases in immunoreactive δ opioid receptors in the hippocampus of rats that consumed ethanol in their diet, whereas μ opioid receptors were decreased in multiple brain regions (118). The disparate results may lie in the method used to measure opioid receptor levels, that is, radioligand binding versus immunohistochemisty. In any case, this topic will continue to receive attention because the endogenous opioid system has been implicated in playing a role in high ethanol consumption and ethanol reinforcement (119, 120) and the opioid receptor antagonist, naltrexone, is approved by the FDA for the treatment of alcoholism (121).

2.6 Summary and Conclusions

Opioid receptor upregulation induced by chronic administration of opioid receptor antagonists is robust and reproducible. Chronic exposure to opioid receptor antagonists increases the number of opioid receptor binding sites without altering receptor affinity. Mu opioid receptors show the largest degree of upregulation following any given dose of naloxone or naltrexone followed by δ opioid receptors, whereas κ receptor are more resilient to antagonist-induced upregulation. Opioid receptor upregulation appears to mediate the behavioral supersensitivity to subsequent opioid receptor agonist administration. There is an increase in potency of morphine and other opioid agonists including etorphine, fentanyl, meperidine, methadone, and oxycodone to produce analgesia following chronic exposure to naloxone or naltrexone. Many other effects of morphine are also increased following chronic antagonist administration including lethality, respiratory depression, inhibition of locus

coeruleus neurons, and stimulation of locomotor activity. The increase in morphine potency on these behavioral and physiological measures following opioid receptor antagonist exposure indicates that the upregulated receptors are fully functional and physiologically relevant. Further, the degree of upregulation of μ and δ receptors parallels the shift in analgesic potency of μ and δ receptor agonists.

Despite the long-held appreciation that opioid receptor antagonists can produce receptor upregulation and functional supersensitivity, elucidation of the molecular mechanisms responsible for this upregulation has proven to be more difficult. Both in brain and in cells lines that express opioid receptors, antagonist-induced opioid receptor upregulation does not appear to be mediated by increases in transcription, as there are no changes in the steady-state levels of opioid receptor mRNAs in response to chronic antagonist treatments. Therefore, it appears that posttranscriptional mechanisms are involved in antagonist-induced opioid receptor upregulation. Recent data generated in cell lines support the hypothesis that opioid receptor antagonists act as pharmacological chaperones that bind to and stabilize newly synthesized or internalized receptors (75, 77, 81, 83). This promotes proper protein folding, maturation, exit from the endoplasmic reticulum, and trafficking to the cell surface. We have shown that κ receptor upregulation is associated with an increase in receptor binding sites and an increase in receptor immunoreactivity; however, upregulation of the δ opioid receptor differs: there is an increase in δ receptor binding sites without a concomitant increase in receptor immunoreactivity. Further work is necessary to elucidate the mechanisms involved in upregulation of the δ receptor.

Drugs other than opioid receptor antagonists can also produce opioid receptor upregulation. It has been established that chronic exposure to cocaine can increase μ opioid receptor number in specific brain regions. This occurs not only in animal models but also in human cocaine abusers. Importantly, PET studies in humans have demonstrated that the level of μ opioid receptor binding in specific brain regions is positively correlated to the degree of craving for cocaine. These results suggest that chronic cocaine use in humans can influence the endogenous opioid system and that these changes may be related to cocaine-induced craving and reinforcement. Ethanol can also produce changes in opioid receptor binding. Studies in animals and cell lines indicate that ethanol can increase delta opioid receptor binding, and studies in alcohol-dependent humans suggest a link between opioid receptor levels and craving for alcohol.

As reviewed in other chapters of this book, opioid receptor antagonists have the potential to be used for a variety of clinical indications. If used chronically, opioid receptor upregulation is a possible sequelae of treatment with such agents. With receptor upregulation, functional supersensitivity to subsequent opioid agonist exposure may occur, and this should be considered when designing treatment regimens for the clinical use of opioid receptor antagonists.

Acknowledgments We thank the editors, Drs. Dean, Bilsky, and Negus, for their efforts in assembling this vast volume of scientific literature. E. M. Unterwald acknowledges Dr. Mary Jeanne Kreek for her role in many of the studies presented herein. R. D. Howells acknowledges the contributions of Kenneth Wannemacher and Prem Yadav to the research performed in his

laboratory. This work was supported in part from grants from NIH/NIDA, P50 DA05130 (MJK), DA09580 (EMU), and DA09113 (RDH).

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