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COMMENTARY

NEUROCHEMICAL CORRELATES OF OPIATE RECEPTOR REGULATION

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The actions of opiates and opioid peptides upon nervous tissue are mediated by μ, δ, K, and σ receptors [for reviews see Refs. 1–3]. The μ receptor is the high affinity site at which morphine-like opiates produce analgesia and a variety of other classical opiate effects. The δ receptor exhibits a higher affinity for the naturally occurring enkephalins (a class of shorter opioid peptides) than for morphine. The K receptor is that site at which ketocyclazocine-like opiates produce analgesia, as well as their unique ataxic and sedative effects. More recently, it has been defined as a receptor highly selective for dynorphin (a 17 amino acid opioid peptide). The σ receptor mediates the psychotomimetic and stimulant effects of SKF-10,047 (*N*-allylnorcyclazocine) and other σ opiates. These receptors exhibit rather different ligand selectivity patterns, are widely distributed in the central nervous system, and exhibit diverse neuroanatomical distributions [4–8]. Findings from studies in neuronal cell lines demonstrating opiate-induced inhibition of adenylate cyclase [9, 10] and in brain and cell lines showing guanyl nucleotide modulation of agonist binding [11–14] make it likely that the μ and δ receptors are coupled to cyclase through an inhibitory guanyl nucleotide binding protein (N_i). It is now well-established that the μ, δ, and K receptors are subserved by three classes of opioid peptides, β-endorphin, the enkephalins, and dynorphin-related peptides. It is, however, unknown whether these receptors represent different gene products or a single gene product which has been post-translationally modified. Moreover, the question arises as to whether they are regulated in a coordinated or an independent manner.

Opiate analgesics are well-known to produce tolerance and dependence *in vivo* and desensitization *in vitro*. The observation of these phenomena has raised the question as to whether opiate receptors undergo up- or downregulation *in vivo*, in response to long-term administration of opiate drugs. Opiate receptor downregulation has been difficult to document. Several groups have reported that chronic administration of narcotic agonists *in vivo* does not produce any change in either receptor number or affinity [15–21]. On the other hand, receptor downregulation has been observed in neurotumor cell lines following long-term exposure to enkephalin [22–24], but not to alkaloid agonists [23]. By contrast, upregulation of brain opiate receptors is well established *in vivo* following denervation [25], and both *in vivo* and *in vitro* following long-term narcotic antagonist administration [21, 26–34].

That brain opiate receptors undergo upregulation is of interest for several reasons. First, long-term administration of antagonist results in a functional supersensitivity to opiates [27–30, 34], as discussed below. Second, the receptor upregulation is accompanied by changes in opioid peptides in specific brain regions [31, 33], an effect which may have implications for regulation of the neuroendocrine system. Third, there are potential clinical ramifications of naltrexone induced opiate receptor supersensitivity both for naltrexone treatment of heroin addicts and also as a possible aid in pain therapy to enhance the analgesic effects of morphine.

This article presents a review of (1) the molecular events associated with opiate receptor upregulation, (2) the nature of opiate functional supersensitivity, (3) the neuroanatomical patterns of receptor upregulation, (4) opioid peptide changes associated with receptor upregulation, and (5) opioid peptide-induced opiate receptor downregulation in culture.

Studies of opiate receptor upregulation

The first reports of antagonist induced opiate supersensitivity and opiate receptor upregulation came from two laboratories. Tang and Collins [27] found that long-term treatment with naloxone results in enhanced morphine-induced analgesia. Because naloxone is rapidly metabolized, it was necessary to prepare rats with indwelling venous cannulas. The same laboratory [32] determined that the enhanced analgesia correlates with an increased number of [^{3}H]naloxone binding sites. Almost simultaneously, Herz and coworkers [28] reported that guinea pigs exposed to naloxone for 1–2 weeks by implantation with naloxone pellets show increased sensitivity to the inhibitory properties of opiates in the isolated ileum preparation. Chronic naloxone treatment also results in increased [^{3}H]etorphine binding in the ileum and brainstem of guinea pigs. It is important to point out that these studies were preliminary reports and, as such, did not control for (1) possible changes induced in endogenous opioid peptide levels that might give rise to *apparent* changes in receptor densities, and (2) the effects of acute antagonist administration.

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Later studies confirmed these findings and showed that chronic, but not acute, administration of the long-acting opioid antagonist naltrexone produces a dramatic increase (+95%) in brain opiate receptor density [26, 29]. In all experiments, brain tissue was incubated at 37° for 30 min and washed by centrifugation before addition of radioligand in order to facilitate the removal of both endogenous ligands and naltrexone. Thus, it is unlikely that the receptor upregulation observed in response to chronic antagonist administration was due to an increased capacity of the same number of active receptors. In experiments designed to target opiate receptor subtypes, it was shown that long-term treatment with naltrexone produces a coordinated upregulation of brain μ and δ receptors but causes no significant change in the density or affinity of K and σ receptors [29]. These findings indicated that the K and σ opiate receptor classes might be subject to independent control mechanisms.

Several neurochemical and functional correlates of opioid receptor upregulation have been found. First, the newly synthesized or unmasked receptors were shown to exhibit an enhanced sensitivity to guanyl nucleotide modulation [26]. Withdrawal from chronic naltrexone treatment resulted in a return to nearly control levels of receptor density and guanyl nucleotide sensitivity in a period of 6 days [29]. These results suggested that upregulation is accompanied by an increased coupling of the receptors to the inhibitory guanyl nucleotide binding protein (N_i), and that downregulation involves the dissociation of the receptor/N_i complex. Second, chronic *in vivo* administration of naloxone or naltrexone was shown to result in enhanced morphine-induced analgesia [27, 29, 34] and an enhanced effect of morphine on neurons of the locus ceruleus [30]. These findings suggest a functional significance for the naltrexone-induced opiate receptor upregulation.

The neuroanatomical pattern of brain opiate receptor upregulation in response to chronic naltrexone administration was elucidated by quantitative receptor autoradiography [31]. The radioligand used was [^{3}H]dihydromorphine, a relatively

Table 1. Optical density measurements of opiate receptors in chronic naltrexone-treated versus control rat brain*

Region	Control brain	Naltrexone-treated brain	% Change
Neocortex			
Layer I	0.11 ± 0.04	0.22 ± 0.02	+100
Layer III	0.17 ± 0.03	0.27 ± 0.01	+59
Subcallosal streak	0.36 ± 0.06	0.31 ± 0.04	−14
Striatum			
Patches	0.38 ± 0.11	0.55 ± 0.02	+45
Other areas	0.22 ± 0.03	0.26 ± 0.04	+18
Nucleus accumbens	0.29 ± 0.06	0.55 ± 0.02	+90
Septum			
Lateral	0.16 ± 0.03	0.29 ± 0.01	+81
Medial	0.28 ± 0.01	0.26 ± 0.01	−7
Amygdala	0.23 ± 0.01	0.51 ± 0.03	+122
Habenular nuclei			
Lateral	0.18 ± 0.01	0.16 ± 0.01	−11
Medial	0.28 ± 0.02	0.53 ± 0.01	+89
Thalamus			
Post. thal. nuc.	0.33 ± 0.02	0.57 ± 0.03	+73
Dorsal thal. nuc.	0.46 ± 0.04	0.61 ± 0.02	+33
Ventral thal. nuc.	0.14 ± 0.01	0.13 ± 0.02	−7
Hippocampus			
Molecular layer	0.34 ± 0.03	0.51 ± 0.02	+50
Other areas	0.19 ± 0.03	0.23 ± 0.02	+21
Hypothalamus			
Ventromedial	0.12 ± 0.02	0.45 ± 0.02	+275
Central gray	0.20 ± 0.01	0.42 ± 0.04	+110
Superficial gray layer of superior colliculus	0.27 ± 0.01	0.50 ± 0.02	+85
Substantia nigra			
Compacta	0.10 ± 0.02	0.30 ± 0.04	+200
Reticulata	0.21 ± 0.02	0.21 ± 0.03	—
Ventral tegmental area	0.12 ± 0.01	0.45 ± 0.02	+275
Locus ceruleus	0.24 ± 0.03	0.21 ± 0.0	−13
Parabrachial nucleus	0.39 ± 0.03	0.54 ± 0.04	+39

* Data are from Ref. 31. Values are means ± S.D., N = 2.

selective μ opioid ligand. Table 1 summarizes the changes in μ opiate receptor density throughout the brain following chronic naltrexone treatment. The largest increases were observed in Layer I of the neocortex, the nucleus accumbens, the amygdala, the ventral tegmental area, the ventromedial hypothalamus and the substantia nigra compacta. Moderate increases were observed in Layer III of the neocortex, the striatum, the lateral septum, the posterior thalamic nucleus and the superior colliculus. No significant changes in density were found in areas surrounding the striatal patches, the medial septum, the ventral thalamic nuclei, the substantia nigra reticulata, or the locus ceruleus.

Are these changes in opioid receptor densities accompanied by changes in any of the opioid peptides? Correlate increases in methionine-enkephalin levels have been documented in two brain regions [31]. The distribution of methionine-enkephalin was determined by radioimmunoassay in selected brain regions following long-term exposure to naltrexone (Table 2). Two dopamine-rich structures, the striatum and the nucleus accumbens, showed significant increases in methionine-enkephalin content after chronic naltrexone treatment (+94% and +40%, respectively; Student's t-test, $P < 0.05$). Moderate increases were detected in the periaqueductal gray and hypothalamic areas. Neocortex did not show a significant change in methionine-enkephalin levels after chronic naltrexone treatment. Antagonist-induced changes in the level of β-endorphin in several brain regions have also been reported [33]. Chronic naltrexone treatment resulted in a decrease in β-endorphin immunoreactivity in the hypothalamus, thalamus and amygdala of the rat. The finding of specific opioid peptide changes raises the intriguing question of whether the peptides themselves or the receptors represent the primary sites of control. The time–course of the peptide changes would need to be determined before this question can be answered. Provocatively, the regions showing the greatest opiate receptor upregulation and increases in endogenous opioid peptide levels following opiate antagonist administration are all dopaminergically innervated regions in which major anatomical and functional interactions between dopaminergic and endogenous opioid peptide neuronal elements have been demonstrated [25, 35, 36]. The possibility thus exists that dopaminergic elements may be involved in the regulation of the endogenous opioid systems.

Other drug and lesion studies have provided further examples of opiate receptor upregulation in adult and neonatal animals. Brunello and co-workers [37] showed that chronic naloxone administration results in a marked increase in the number of μ and δ receptors in the striatum and brainstem of C_{57} BL/6J mice, but produces no change in methionine-enkephalin levels. The increase in receptor number was accompanied by an enhancement of morphine-induced locomotor activity. Specific brain lesions have also been shown to produce opiate receptor upregulation. For example, our laboratory [25] found that injection of the neurotoxin 6-hydroxydopamine into the lateral substantia nigra leads to an increase in δ receptors in the striatum. Simantov and Amir [38] reported that monosodium glutamate (MSG)-induced lesions of the arcuate nucleus of the hypothalamus leads to an increase in μ receptors in the midbrain of neonatal mice. Receptor upregulation was correlated with an enhanced response to morphine and naltrexone in tests of the intact animal for thermal pain sensitivity. By contrast, MSG administration to neonatal rats produced a selective increase in the number of δ receptors in the thalamus, with no apparent change in μ receptors or in δ receptors of other brain regions [39]. More recently, Holaday and co-workers [40] have shown that repeated electroconvulsive shock treatment produces significant increases (>5%) in opiate receptor density as measured by [^{3}H]diprenorphine binding in specific brain regions of the olfactory bulb, nucleus accumbens and the caudate nucleus. Chronic administration of opiate antagonists to neonatal rats or indirect exposure *in utero* also leads to alterations in opiate receptor development. Bardo and co-workers [21] showed that rat pups treated chronically with naloxone exhibited significant increases in opiate receptors of the hypothalamus, striatum and neocortex [21]. These antagonist induced receptor increases were accompanied by supersensitivity to morphine in analgesia paradigms and were reversed following cessation of the antagonist treatment.

Upregulation studies in vitro

The upregulation of opiate receptors has also been observed *in vitro* using explant cultures of fetal mouse spinal cord with attached dorsal root ganglia (DRG). The advantages of studying opiate receptor regulation in cultured cells are 2-fold. First, it is possible to control rather precisely the physiochemical environment of the cultures. Second, it is possible to correlate receptor changes with changes in cellular function. Particular questions which can be addressed in such systems are: (1) Does the

Table 2. Regional distribution of met-enkephalin after long-term exposure to naltrexone*

	Methionine-enkephalin (ng/mg protein)		
	Naltrexone-treated	Control	% Change
Neocortex	10.0 ± 0.6	11.8 ± 1.5	−15
Hypothalamus	9.5 ± 0.1	7.3 ± 0.8	+30
Periaqueductal gray	9.6 ± 0.3	7.6 ± 0.7	+26
Nucleus accumbens	11.6 ± 0.5	8.3 ± 0.8	+40†
Striatum	18.4 ± 2.1	9.5 ± 1.0	+94†

* Data are from Ref. 31. Values are means ± S.E.M., N = 4.

observed increase in opiate receptor levels represent the synthesis of new receptors or the activation (or unmasking) of previously existing receptors? (2) Does upregulation require the presence of synaptic connections or does it occur in dissociated membranes?

Cultures of spinal cord with attached DRG develop high levels of stereospecific opiate receptors, especially in the DRG neurites [41]. Chronic exposure of the explant cultures to naloxone (10 μM, 7 days) was found to produce a 51% increase in μ opiate receptor density relative to control cultures [42]. The antagonist action was shown to be stereospecific, as it was produced by (−)- but not by (+)-naloxone and was dose dependent. Half-maximum naloxone-induced receptor upregulation occurred at 2 days; receptor density was maximal at 5 days. Exposure of the explant cultures to naloxone (10 μM) in the presence of the protein synthesis inhibitor cycloheximide (1 μM; a concentration which blocks >90% protein synthesis) resulted in receptor density changes that were similar to those observed in cultures exposed to naloxone alone. This finding suggests that antagonist-induced opioid receptor upregulation does not require the synthesis of new receptor molecules.

To determine whether upregulation of this type can occur in the absence of the formation of synaptic connections, isolated explants of DRGs or cord were grown in the presence or absence of naloxone [43]. Isolated DRG explants exhibited a more pronounced antagonist-induced increase in receptor number than did DRG-cord explants. This finding indicates that antagonist-induced upregulation of opiate receptors can occur in peripheral DRG neurons in the absence of synaptic connections to their target tissues. It also suggests that regulation of these receptors on isolated DRG neurons may be more plastic.

Studies of opiate receptor downregulation

In contrast to the case of opiate receptor upregulation, opiate receptor downregulation following chronic agonist treatment *in vivo* has been difficult to document. Earlier studies failed to show any systematic change in receptor number [16–21]. Davis and co-workers [44] showed that the development of tolerance to morphine was accompanied by a reduction of opiate binding in the brainstem slice preparation. Homogenization was carried out *after* ligand incubation in this preparation, a procedure which may be important for maintaining membrane–receptor integrity. More recently, however, Holaday and co-workers [40] reported a 17–20% increase in opiate receptor density following chronic morphine treatment.

Opiate receptor dowregulation has been documented in cultures of neurotumor cells. Cultured mouse neuroblastoma cells (N4TG1) and neuroblastoma-glioma hybrid cells (NG108-15) have been shown to bear exclusively δ receptors [4]. Early studies involving the hybrid cell line indicated that occupation of these receptors by opiates or opioid peptides leads to a time-dependent inhibition of adenylate cyclase activity [10, 45]. Continued exposure of the cells to agonists leads to a return to normal of adenylate cyclase activity; removal of opioids leads to an increase above normal levels, a phenomenon thought to resemble tolerance and dependence in animals. Cuatrecasas and co-workers [24] used a rhodamine derivative of enkephalin to label opiate receptors in cell culture; visualization of the receptors by fluorescence microscopy provided evidence that they slowly form clusters, but do not appear to internalize. This finding is in contrast to many other polypeptide hormone receptors which do internalize; the internalization process is thought to be related to downregulation or densensitization.

More recently, several laboratories [22–24, 46] have shown that long-term exposure of neurotumor cell-lines to methionine-enkephalin results in a decrease in receptor density. Chronic exposure to opiate agonists, however, had no effect [23]. Studies of [^{3}H]DADLE (D-Ala^2, D-Leu^5-enkephalin) uptake by N4TG1 cells [22] indicate that enkephalin is internalized via receptor-mediated endocytosis. One possible explanation is that opioid peptides bind differently than do opioid narcotic agonists and thereby produce differing effects on the same receptor.

Conclusions

In conclusion, we have reviewed studies of the neurochemical correlates of opiate receptor up- and downregulation. Several pressing issues still remain to be resolved. Why is opiate receptor upregulation induced *in vivo*, whereas downregulation is not? Is the molecular mechanism underlying downregulation the mirror-image of that underlying upregulation? Do opioid peptides bind differently than do opioid agonists to the same receptor and thereby elicit downregulation *in vitro*? Is the synthesis of the opioid peptides regulated by long-term administration of opiate agonists or antagonists?

One possible model to explain the findings to date is that active opiate receptors in the absence of drug treatment exist in a dynamic equilibrium with a nearly equal concentration of inactive, "silent" receptors. Long-term exposure of the system to opiate antagonists leads to the activation or unmasking of the "silent" receptors, giving rise to an apparent upregulation. Long-term exposure of the system to opiate agonists or opioid peptides might lead to coupling of the receptors to cyclase, followed by internalization. As active receptors disappear, these would be replaced by the conversion of "silent" receptors to active ones. In the case in which activation paralleled internalization, no apparent change in receptor density would be observed. Only in the case in which internalization exceeded reactivation (i.e. opioid peptides) would apparent downregulation occur. The findings from our laboratory involving a cell culture system are consistent with such a model. Future experiments should be aimed at an understanding of how peptides produce receptor downregulation *in vitro* and of how opioid peptides might regulate their respective receptors.

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