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PERSPECTIVE

Regulation of Opioid Receptor Function by Chronic Agonist Exposure: Constitutive Activity and Desensitization

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Opium poppy extracts have been used for thousands of years to control pain, gut motility, and mood. However, chronic use of opiates can lead to tolerance, withdrawal, dependence, and addiction. Decades of study have established that the changes caused by sustained opioid receptor activation are complex and multifaceted. At the systems and behavioral levels, these changes are evident as compensatory adaptations within the neuronal circuits and adaptive learning. At the cellular level, changes in gene expression and opioid responses are evident. At the molecular level, sustained opiate receptor activation leads to changes in the efficiency of G protein activation and agonist efficacy.

Considerable progress in the molecular understanding of opioid receptor functioning followed the initial cloning of the μ-opioid receptor (MOR) (Chen et al., 1993; Fukuda et al., 1993; Wang et al., 1993). Detailed analysis of the sites in MOR responsible for ligand binding, G protein coupling, and phosphorylation-induced desensitization has emerged. For example, extensive point-mutation analysis of MOR has provided insight into the residues required for ligand affinity and selectivity (Table 1). Competition binding assays with MOR point mutants have shown that ligand binding affinity is influenced by over 20 amino acids, notably MOR D114 for full agonist binding and MOR H297N for partial agonist and antagonist binding (Surratt et al., 1994; Bot et al.; 1998; Xu et al., 1999b). Despite the involvement of numerous amino acids in conveying MOR-ligand affinity, MOR selectivity seems highly dependent on four amino acids: D128, N150, K303, and W318 (Surratt et al., 1994; Mansour et al., 1997; Xu et al., 1999a; Bonner et al., 2000; Larson et al., 2000).

Sites within MOR responsible for G protein coupling have also been defined (Table 2). The image that emerges is one of rich complexity in which receptor functioning is highly regulated at many checkpoints. Although much of this complexity is consistent with previously characterized G protein-coupled receptors, the resulting understanding of opioid receptor regulation provides an essential foundation for further studies of opioid tolerance and addiction. In addition, the underlying molecular mechanisms can begin to be defined for certain poorly understood phenomena. One example of this is the constitutive activity of opioid receptors described by Liu and Prather (2001) presented in this issue.

Constitutive activity for other G protein-coupled receptors (GPCR) has been observed previously (see Lefkowitz et al., 1993), and constitutive activity for MOR has also been demonstrated (Wang et al., 1994, 2000). However, the basis for constitutive GPCR activity is not known. In general terms, receptor theory suggests that agonist binding shifts the receptor from the basal state (having negligible or low rate of G protein activation) to a ligand-bound, activated state (having a high rate of G protein activation). As these hypothetical states have not been visualized, a clear description of intrinsic efficacy is not yet available. Nevertheless, the constitutively active state is presumably a stabilized form of the receptor that does not require agonist binding to maintain a conformation necessary to produce detectable G protein activation. The important question is what kind of post-translational modifications (e.g., changes in phosphorylation or accessory protein binding) stabilize this conformation of the receptor.

Chronic exposure to opiates produces the constitutively activated state by mechanisms that are not yet clear. Sadee and colleagues present the interesting hypothesis that constitutive activation of MOR results from an H7-sensitive phosphorylation event (Wang et al., 1994) that reduces a tonic inhibition of receptor coupling caused by calmodulin binding to the 3rd intracellular loop of MOR. Because this same loop has been shown to regulate constitutive activity of the dopamine receptors (Charpentier et al., 1996) and has been shown to be important for MOR association with G proteins (Table 2), the hypothesis is plausible.

ABBREVIATIONS: MOR, μ-opioid receptor; GPCR, G protein coupled receptor; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]-enkephalin; GRK, G protein receptor kinase; HEK, human embryonic kidney.

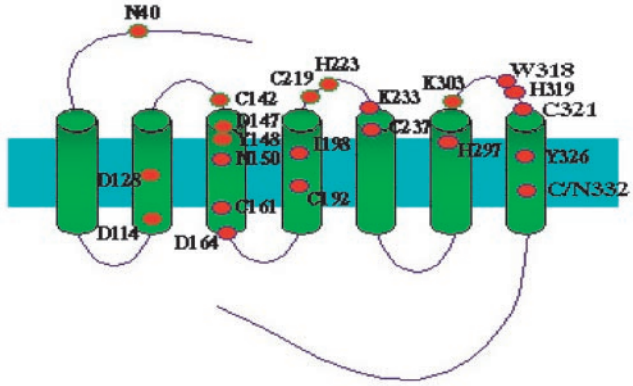
Clues to the nature of constitutive activity may also be revealed by a consideration of the sites in MOR found by point-mutation analysis to affect intrinsic efficacy (Table 2). Mutation of MOR S196L and MOR H297N changed the antagonist naloxone into an agonist, and naloxone activation of the mutant receptors produced DAMGO-like inhibition of forskolin-stimulated adenylyl cyclase activity in Chinese hamster ovary cells and activation of potassium channels in *Xenopus laevis* oocytes (Claude et al., 1996; Spivak et al., 1997; Spivak and Beglan, 2000). Alterations in agonist intrinsic efficacy were likewise produced by point mutations at specific tyrosine residues in the putative cytoplasmic face of MOR, suggesting that Y106F and Y166F are also sites that regulate G protein coupling (see Table 2).

Of particular interest is the construction of constitutively active mutant MOR D164Q receptors that displayed enhanced basal guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate binding and constitutive, spontaneous internalization and down-regulation without agonist in Chinese hamster ovary cells (Li et al., 2000). The MOR D164Q and Y166F point mutant data provide evidence that highlight the role of the highly conserved DRY motif in MOR coupling to and activation of G proteins. Support for this hypothesis has been generated in other receptor systems (Valiquette et al., 1995; Scheer et al.,

TABLE 1

Site mutations affecting ligand binding

Summary of opioid ligand binding experiments with point mutated μ-opioid receptors. Schematic summarizes point-mutated amino acids in the MOR from 17 studies. Table summarizes results of competition binding assays with assorted opioid ligands using the expressed point-mutated MOR protein.



Receptor Mutation	Effect	Expression System	Citation
N40D	Increases affinity for β-endorphin 3-fold	AV-12	Bond et al., 1998
D114A/N	Reduces binding affinities for full agonists	COS/HEK293/CHO	Surratt et al., 1994; Bot et al., 1998; Xu et al., 1999b
D128A	Decreases agonist binding to low-affinity state	COS	Befort et al., 1996
D128N	Blocks diprenorphine binding; decreases DOR-selective peptide and alkaloid affinity	COS	Befort et al., 1996
C142A/S	Blocks opioid binding possibly by disruption of disulfide bond	CHO	Zhang et al., 1999
D147A/N	Reduces affinity of naltrexone, MOR and DOR agonists	COS & CHO	Befort et al., 1996; Li et al., 1999
Y148F	Reduces binding affinity of fentanyl derivatives but not DAMGO or naloxone	COS	Xu et al., 1999a
N150A	Increases binding affinity of MOR, KOR, and DOR agonists but not antagonists	COS	Mansour et al., 1997
D164Q	Blocks diprenorphine binding	HEK293/CHO	Li et al., 2000
C161S, C192S, C237S, C321S and C332S	Blocks irreversible antagonist binding	COS/HEK293	Deng et al., 2000; Xu et al., 2000
I198V	Reduces affinity of morphine and DAMGO 4- to 5-fold	COS	Mansour et al., 1997
C219A/S	Blocks opioid binding possibly by disruption of disulfide bond	CHO	Zhang et al., 1999
H223S	Protects against NEM inactivation, decreases affinity for bremazocine	HEK293	Shahrestanifar et al., 1996
K233A/R/H/L	Eliminates irreversible binding of β-FNA	CHO	Chen et al., 1996
H297A	Eliminates [^{3}H]DAMGO, EKC, and bremazocine binding	COS	Mansour et al., 1997
H297N	Reduces affinity for partial agonists and antagonists, but not full agonists	HEK293	Bot et al., 1998
K303E	Confers affinity for KOR-selective antagonist, Nor-BNI	COS	Larson et al., 2000
W318A/L/K	Reduces selectivity for MOR ligands; confers increased affinity for DOR-selective ligands	COS	Xu et al., 1999a; Bonner et al., 2000; Ulens et al., 2000
H319A	Reduces affinity for numerous opioid ligands, but has no effect on naloxone or bremazocine	COS	Xu et al., 1999b
Y326F	Decreases affinity for a “wide spectrum” of opioid ligands	COS	Mansour et al., 1997
N332D	Eliminates binding of DAMGO or diprenorphine	CHO	Xu et al., 1999b
D114N+N332D	Restored high-affinity binding for antagonists; partially restored binding affinities of morphine and DAMGO	CHO	Xu et al., 1999b

1996; Rhee et al., 2000), and should reveal important information about opioid receptor activation as studies mutating MOR R165 and corresponding structures in the other opioid receptors become available. Together these findings suggest that alterations in the conformation of the cytoplasmic face of MOR, either by tyrosine phosphorylation of the DRY motif or changes in the 3rd intracellular loop, stabilize a receptor configuration able to more efficiently activate G proteins.

A second issue of particular interest touched on by Liu and Prather (2001) is the concept that morphine has 'special' properties that distinguish it from other opioid agonists. The idea was initially derived from studies of opioid receptor internalization in cell lines (Keith et al., 1996). Strong opioid agonists possessing high intrinsic efficacies (e.g., etorphine, DAMGO, fentanyl) caused rapid receptor internalization and receptor desensitization whereas weak partial agonists such as morphine did not. This difference was initially attributed to differences in agonist efficacy because the G protein receptor kinase (GRK) and β-arrestin machinery responsible for receptor desensitization and internalization is activated by G$\beta\gamma$. Thus agonists with higher efficacies would be expected to more effectively activate this machinery. Yu et al. (1997) and Whistler et al. (1999) noted that MOR phosphorylation and desensitization caused by a series of opioid agonists did not exactly correlate with agonist efficacy. In particular, morphine was found to have a low propensity to produce tolerance, whereas two agonists with lower efficacies (methadone and LAAM) more readily produced tolerance.

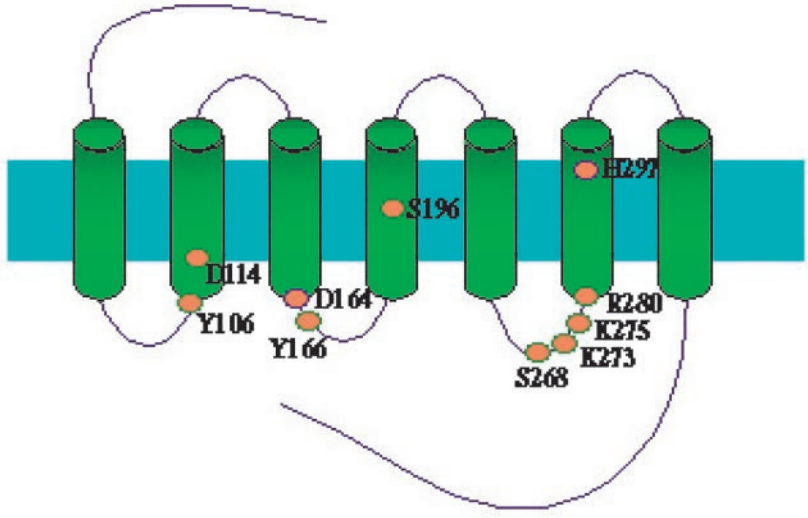
Whistler et al. (1999) propose that the inability of morphine to promote receptor desensitization leads to sustained receptor activation; thus, heightened compensatory changes ultimately lead to the development of tolerance and dependence. Furthermore they provide evidence for the dissociation of agonist efficacy and regulation of MOR by GRK and arrestin. This is an exciting proposal and lends support to the possibility of developing MOR agonists that are effective in producing analgesic effects with a decreased tendency to promote tolerance. Whistler et al. (1999), propose deriving a RAVE index for opioid agonists that may better predict the ability of opioid agonist to promote tolerance by expressing the relative ability of agonists to activate MOR and drive receptor endocytosis as a ratio. This hypothesis partially motivated the analysis of chronic morphine actions performed by Liu and Prather (2001).

The induction of tolerance and receptor desensitization by chronic morphine exposure have been studied extensively. Although the molecular mechanisms are not completely understood, the recent finding that disruption of the β-arrestin2 gene in mice greatly decreases morphine tolerance offers a significant demonstration of the importance of the present model of GRK and arrestin regulation of opioid receptors in vivo (Bohn et al., 2000). According to the present model,

TABLE 2

Site mutations affecting G protein coupling efficiency

Summary of alterations in opioid intrinsic efficacy produced by point mutation of the μ-opioid receptor. Schematic summarizes point-mutated amino acids in the MOR from 13 separate studies. Table summarizes observed changes in opioid intrinsic efficacy using the expressed point-mutated MOR protein in functional assays.



Receptor Mutant	Effect	Expression System	Citation
D114A/N	Reduces agonist efficacy	Neuro2A/CHO	Chakrabarti et al., 1997; Xu et al., 1999b
Y106F, Y166F	Blocks increase in intrinsic efficacy of DAMGO induced by insulin	*X. laevis* oocytes	McLaughlin and Chavkin, 2001
D164Q	Confers constitutive activity	HEK293/CHO	Li et al., 2000
S196L	Confers agonist properties to antagonists	CHO, *X. laevis* oocytes	Claude et al., 1996
S268P	Allelic variation of hMOR results in a "weaker but persistent coupling"	HEK293, *X. laevis* oocytes, COS	Koch et al., 2000; Befort et al., 2001
K273A,R275A	Renders MOR insensitive to calmodulin; increases intrinsic efficacy	HEK293	Wang et al., 1999; Wang et al., 2000
R280L	Decreases intrinsic efficacy of DAMGO	HEK293	Wang, 1999
H297A/N/Q	Confers agonist activity to naloxone; increases intrinsic efficacy of partial agonists	COS, *X. laevis* oocytes	Surratt et al., 1994; Spivak et al., 1997; Spivak and Belgian, 2000

agonist occupation of the receptor promotes GRK phosphorylation of MOR, leading to the activation of arrestin and formation of an inactive receptor arrestin complex. Interestingly, precise characterization of the desensitization process in simpler expression systems has proven to be quite elusive and has led to a large body of apparently contradictory reports concerning the specifics of cellular models of opioid tolerance. Table 3 summarizes findings from many laboratories and multiple expression systems implicating specific residues important for agonist dependent down-regulation or desensitization of MOR.

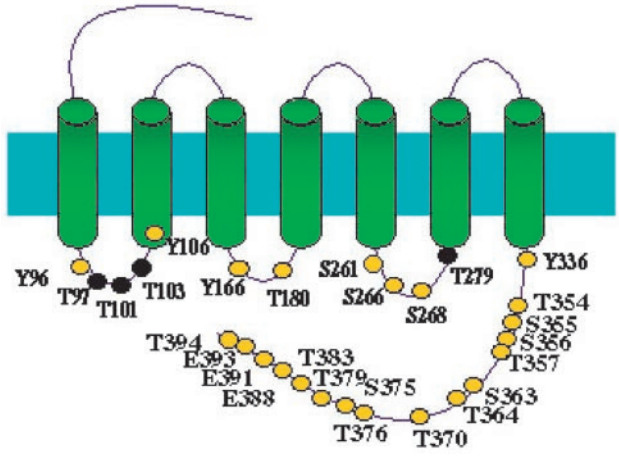
Recent reports have gone a long way in resolving some of the discrepancies by considering the cyclic nature of the GRK and arrestin regulation of MOR. For example, alanine substitution of the most terminal threonine of the rat μ-opioid receptor has been implicated in reducing agonist dependent desensitization (Pak et al., 1997). However, both the splice variant of MOR lacking this putative GRK phosphorylation site and the MOR mutant having alanine substitution at this site progressed through the internalization and resensitization cycle at a much faster rate. The significantly increased kinetics of resensitization made this receptor mutant seem to lack agonist dependent desensitization (Koch et al., 1998; Wolf et al., 1999). With the same theme, Law et al. (2000) carefully examined the role of receptor recycling in an attempt to resolve the findings that rapid receptor phosphorylation did not seem to correlate with the much slower changes in receptor desensitization observed in many systems (Capeyrou et al., 1997; El Kouhen et al., 1999). Here, either blockade of receptor internalization or reduction in functional receptor with the irreversible MOR antagonist β-funaltrexamine dramatically increased the MOR desensitization rate and greatly increased the correlation between desensitization and phosphorylation. As demonstrated by Wolf et al., (1999) and Law et al. (2000), subtle changes in the kinetics of receptor recycling can have dramatic effects on the apparent coupling efficiency of MOR upon prolonged agonist treatment. Hence, if these points are considered more carefully, it is likely that the specifics of GRK and arrestin regulation will be more readily compared between expression systems.

Chronic agonist exposure thus seems to evoke the opposing processes of receptor desensitization and constitutive receptor activity. This paradox needs better resolution. Interestingly, Liu and Prather (2001) found that chronic exposure to morphine or DAMGO were both able to induce constitutive

TABLE 3

Site mutations affecting phosphorylation, internalization, and desensitization

Summary of alterations in opioid phosphorylation, internalization, and desensitization produced by point mutation of the μ-opioid receptor. Schematic summarizes point-mutated amino acids in the MOR from 10 separate studies. Table summarizes observed changes in opioid phosphorylation, internalization, and desensitization using the expressed point-mutated MOR protein in functional assays.



Receptor Mutation	Effect	Expression System	Citation
S363A/T370A	Loss of basal phosphorylation	HEK293	El-Kouhen et al., 2001
T370A/S375A	Loss of agonist-dependent phosphorylation	HEK293	El-Kouhen et al., 2001
T180A	Loss of GRK3/arr3 dependent uncoupling	*X. laevis* oocytes	Celver et al., 2000
T354/S355/S356/T357	Required for agonist-dependent desensitization	HEK293	Wang 2000
S356A/S363A	Loss of agonist-dependent internalization	Neuro2A/HEK293	Burd et al., 1998
T383	Required for complete agonist-dependent desensitization	CHO	Deng et al., 2000
T394A	Required for agonist-dependent phosphorylation and desensitization	CHO/HEK293	Deng et al., 2000; Koch et al., 1998
Y96F/Y106F/Y166F/Y336F[a]	Blocks genistein sensitive internalization	CHO	Pak et al., 1999
T394A	Enhanced receptor recycling	HEK293	Wolf et al., 1999
S266P[a]	Loss of calmodulin kinase II-dependent desensitization	HEK293	Koch et al., 1998
T364-T383A	Partial block of agonist-dependent desensitization	CHO	Pak et al., 1999
S261A/S266A	Loss of calmodulin kinase II-dependent desensitization	HEK293; *X. laevis oocytes*	Koch et al., 1997
Truncation at T354	Constitutive internalization	HEK293	Segredo et al., 1997
E288Q/E391Q/E393Q	Loss of agonist-dependent internalization	CHO	Pak et al., 1997

[a] Homologous residue in rat MOR (GenBank accession L130369).

activity proportional to their drug efficiacies. This proposal is consistent with reports that GRK and arrestin regulation of GPCRs is directly correlated with agonist efficacy (Kovoor et al., 1998; Szekeres et al., 1998). In this highlighted report (Liu and Prather, 2001) and in other systems, it is clear that morphine is a partial MOR agonist. Thus, the contribution of receptor reserve and the role of agonist efficacy in controlling tolerance induction rates need careful consideration. For example, the RAVE model requires a better measure of intrinsic efficacy (e.g., in the absence of spare receptors), correction for constitutive activity, and an assessment of receptor resensitization (i.e., recycling) rates. Without those additional measures, it seems premature to discount the role of agonist efficacy and to ascribe to morphine "special" properties.

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