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Chaperone-like effects of cell-permeant ligands on opioid receptors

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1. ABSTRACT

The number of cell surface opioid receptors reflects a delicate balance between biosynthesis pathway and endocytosis pathway. The post-activation endocytic events such as internalization, recycling and degradation have been well-documented; however, only a few studies have been conducted on the regulatory events occurring along the protein biosynthesis pathway, including protein folding, endoplasmic reticulum (ER) export, ER-associated degradation, vesicular trafficking and membrane targeting and insertion. Accumulated *in vitro* evidence has demonstrated that expression of the opioid receptors, either wild-type or mutated, is subject to regulation by prolonged treatment with cell-permeant ligands that exert their regulatory effects post-transcriptionally. These hydrophobic ligands, both agonists and antagonists, were found to act in the ER like ER-resided molecular chaperones to positively affect stability, folding efficiency and/or ER export rate of newly-synthesized receptor proteins. Moreover, a number of observations demonstrated that long-term opioid antagonists up-regulated the receptors *in vivo,* in accord with the *in vitro* findings. Potential therapeutic applications of the chaperone-like function of opioid ligands are discussed.

2. INTRODUCTION

There are at least three types of opioid receptors (OPRs), mu, delta and kappa. They are members of rhodopsin sub-family of the seven-transmembrane receptor (7TMR) superfamily. After agonists bind to the receptor proteins in plasma membranes, which induces conformational changes, $G_{i/o}$ protein-dependent and -independent signaling cascades were initiated (1). The capacity of opioids to modulate downstream signaling molecules is dependent on the availability of the receptors in active conformations on the cell surface. The number of cell surface receptors can be regulated through both agonist-induced post-activation endocytosis pathway and protein biosynthesis pathway, reflecting a balance between cellular events including endocytosis, export and degradation (2-4).

Agonist-induced adaptative events of the OPRs have been extensively investigated and well documented (2,5). Following activation by an agonist, the OPRs are phosphorylated by G protein-coupled receptor kinases and then non-visual arrestins are recruited which reduces coupling between the receptor and G protein, causing receptor desensitization. Through a clathrin- and dynamin-

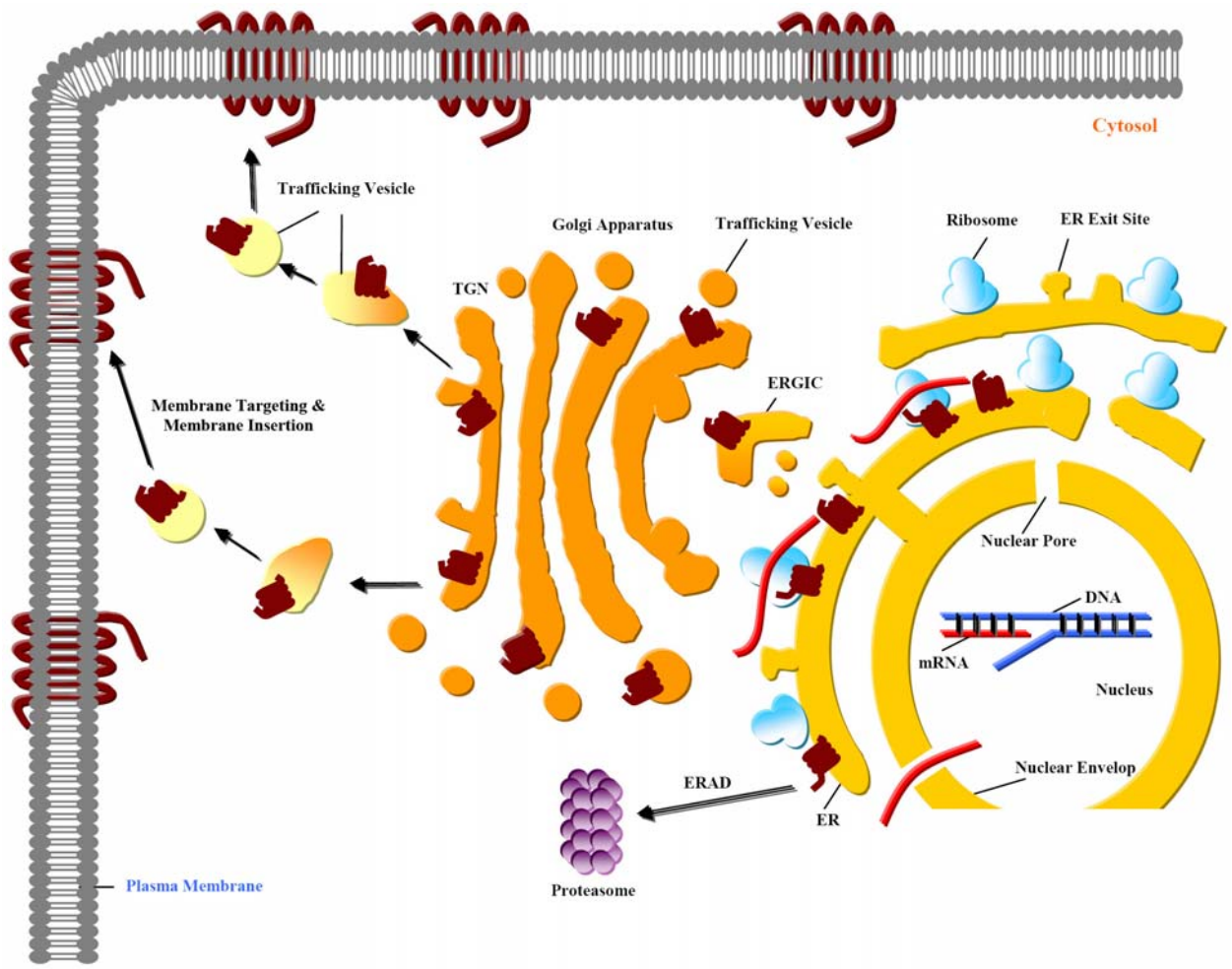

Figure 1. Biosynthesis Pathway of OPRs**.** Following DNA transcription in the nucleus, mRNA transcript of the receptor is transported to ribosome-associated ER for protein translation. The newly-synthesized receptor proteins assuming natively-correct conformation leave ER for Golgi apparatus via ERGIC and then target to plasma membrane from TGN through vesicular trafficking for membrane insertion.

dependent pathway, phosphorylated OPRs are endocytosed (internalization) followed by either dephosphorylation and recycling (resensitization) or degraded via both lysosome and proteasome systems (down-regulation). Along this post-activation endocytosis pathway, therefore, there are several sites such as internalization, recycling and down-regulation playing roles in regulating expression level of cell surface OPRs. Recently a few studies have demonstrated regulation of OPRs by ligands that influence the protein biosynthesis pathway and thus affect receptor expression on plasma membranes as well, which is the focus of this chapter.

3. PROTEIN BIOSYNTHESIS PATHWAY AND ENDOPLASMIC RETICULUM

Biosynthesis of membrane-bound receptors is similar to that of other cell surface proteins. The genes are transcribed in the cell nucleus to generate mRNAs, which are subsequently translocated into cytosol and then translated into receptor proteins in ribosome-associated ER. From the ER, the newly-synthesized receptor proteins are exported to ER-Golgi intermediate compartment (ERGIC) and then to cis-Golgi apparatus followed by Golgi cisternae and trans-Golgi network (TGN), where the newly-synthesized receptors are transported to plasma membrane for membrane targeting and insertion via the anterograde vesicular trafficking pathway (3). As a consequence, factors impacting gene transcription, mRNA translation, ER or Golgi export, vesicular trafficking, and membrane targeting and insertion may change receptor level at the cell surface (Figure 1).

As the first compartment responsible for post-translational processing of 7TMR proteins, the ER plays very prominent roles in controlling their fates (Figure 2). ER has been widely accepted as the quality control system of newly-synthesized proteins in cells on the basis of its ability to affect protein glycosylation, folding, ER-to-Golgi export and retro-translocation of misfolded proteins into

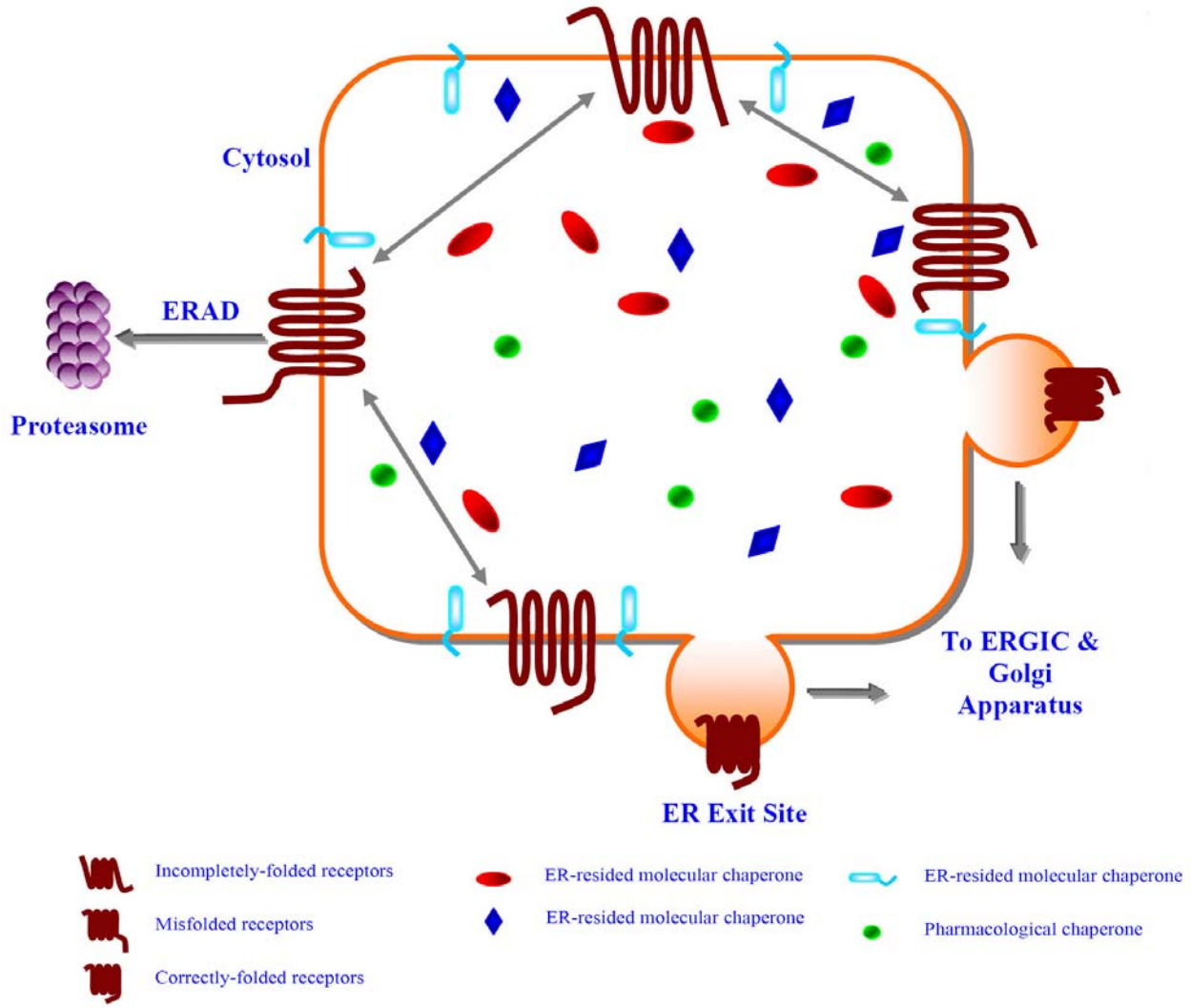

Figure 2. Pharmacological chaperones facilitate folding of 7TMRs in the ER and thus enhance their export to ERGIC. Following mRNA translation, the nascent polypeptide products bind co-translationally to molecular chaperones which facilitate folding. If they achieve natively-correct conformations, newly-synthesized receptors are exported to ERGIC for further anterograde intracellular trafficking. If they are still misfolded after cycles of interaction with the molecular chaperones, the receptors are delivered to proteasomes for ERAD. In the presence of pharmacological chaperones, incompletely-folded/misfolded conformers can reach their natively-correct conformations more readily and rapidly, thereby enhancing ER export and expression of 7TMRs on the plasma membrane.

cytosol for degradation (6-8). Following mRNA translation, the nascent polypeptide products bind to folding-facilitating ER-resided molecular chaperones (including calnexin, calreticulin and BiP) co-translationally to form incompletely-folded intermediate conformers. The binding is through hydrophobic contacts or mediated by N-glycans added to the new polypeptides in the ER. These chaperone proteins can retain the unfolded or incompletely-folded receptor intermediates in the ER and give them time to go through the ER-folding cycles to reach correct folding. Nevertheless, if the newly synthesized proteins ultimately fail to fold correctly, they are translocated into cytosol via translocons, ubiquitinated and degraded in proteasomes via the ER-associated degradation (ERAD) pathway. Therefore, adopting a natively correct conformation is the prerequisite for cell surface receptors to exit from this quality control system and eventually to reach their action sites on the plasma membrane. On the other hand, protein misfolding may result in diminished or deficient cell surface expression and function of the receptors.

4. DISEASES CAUSED BY MISFOLDING OF MUTATED CELL SURFACE PROTEINS

In recent years, many human inherited diseases have been shown to be linked to the ER retention and/or the subsequent ERAD of misfolded proteins that under normal circumstance function on the plasma membrane, such as

cystic fibrosis (cystic fibrosis transmembrane conductance regulator), long QT syndrome type 2 (*ether-a-gogo*-related gene K^+ channel), nocturnal frontal lobe epilepsy (nicotinic acetylcholine receptor $Alpha_4$ subunit) (9-11) (for a review, see (12)).

Several 7TMR mutants are also involved in ER retention-related human inherited diseases. Mutations of vasopressin V_2 receptor, gonadotropin-releasing hormone receptor (GnRHR) and rhodopsin, which induce improper folding, are linked to nephrogenic diabetes insipidus, hypogonadotropic hypogonadism and retinitis pigmentosa, respectively (13-16).

5. CHAPERONES

Mutations of proteins result in ER retention because these mutations impose impassably thermodynamic barrier for adopting proper folding and thus resulting in production of misfolded conformers that are not exported efficiently from ER and contribute to the loss-of-function clinical phenotypes (17). If the mutations are outside of the regions involved in its activity, which is true in most cases, agents that function like molecular chaperones to promote formation of the export-competent conformers and thus ER-to-Golgi export of proteins would be useful. The compounds may rescue membrane trafficking, surface expression and signal transduction of the receptors.

A group of low-molecular-weight compounds, acting like the ER-resided molecular chaperones to stabilize the improperly folded proteins, are effective in mitigating ER retention of the proteins associated with the above mentioned diseases. These compounds are known as chemical chaperones, including glycerol, trimethylamine N-oxide, dimethylsulfoxide, proline and 4-phenylbutyric acid (17,18). However, lack of specificity and high concentrations required make these compounds impractical as therapeutic agents (17,19).

Another type of small molecules, which specifically bind to the proteins of interests, have been employed in preclinical as well as clinical studies to stabilize native conformation, promote ER-to-Golgi export, up-regulate cell surface expression and control aforementioned genetic diseases (20-22). In 2000, Morello *et al.* (14) demonstrated that cell-permeant antagonists caused up-regulation of cell surface V_2 vasopressin receptor mutants through binding and stabilizing the misfolded receptor proteins and named these agents as pharmacological chaperones for the first time. The term pharmacological chaperone has been extended to include selective ligands, substrates and inhibitors of the cognate unstable ER-retained receptors, enzymes and transmembrane channels, respectively. The concentrations of the pharmacological chaperones required to facilitate the export of their target proteins from the ER are usually sufficiently low to be therapeutically approachable, thus with low propensity for serious adverse effects (13,17,19).

Misfolding-caused inefficient ER export and loss of expression/function of cell surface receptors is observed not only for some mutated 7TMRs, but also for some wild-type 7TMRs (11,19,23,24). Therefore, pharmacological chaperones can target wild-type 7TMRs to facilitate adoption of native conformation, promote ER export and enhance cell surface expression. Pretreatment with cell-permeant selective antagonists significantly increase the maturation efficiency (i.e. surface expression) of wild-type histamine H_2, $beta_2$-adrenergic, gonadotropin releasing hormone, dopamine D_4, vasopressin V_2 and kinin B_1 receptors (25-30) (for a review, see (31)).

6. PHARMACOLOGICAL CHAPERONE EFFECTS OF CELL-PERMEANT LIGANDS FOR OPRS

Petaja-Repo *et al.* (32) found that the wild-type human delta opioid receptors (hDOPRs) expressed in HEK-293S cells were detected as immature (precursor) and mature forms, which differed in the extent of glycosylation, and that only ~ 40% of the precursor receptor was converted to the mature form and expressed on the plasma membrane. The majority of hDOPRs were misfolded or incompletely-folded, retained in the ER and some were retro-translocated into cytosol, ubiquitinated and degraded in the proteasomes via the ERAD pathway. Therefore, it was suggested that folding and ER export of newly-synthesized receptors were the rate-limiting steps along the hDOPR protein biosynthesis pathway and that factors impacting folding, ER-to-Golgi exit and ERAD may regulate maturation and expression of hDOPRs. Subsequently, the same group (23) reported that ligands of hDOPRs, either agonists or antagonists, enhanced maturation extent of the wild-type receptors and cell-permeability was necessary for the ligands to up-regulate hDOPR cell surface expression. In addition, the D95A-hDOPR mutant was poorly expressed on cell surface and prolonged treatment with hydrophobic hDOPR agonists and antagonists promoted its maturation and cell-surface expression. These authors suggested that these ligands functioned as pharmacological chaperones to affect positively receptor folding and membrane expression. This is the first report of chaperone-like effects of ligands on the expression of opioid receptors.

In 2001, Li *et al.* (33) found that 72-h treatment with 20 microM naloxone up-regulated by 45% the wild-type rat mu opioid receptors (rMOPRs) stably expressed in CHO cells, but with no influence on its mRNA level, indicating that naloxone exerted its effects post-transcriptionally. However, it was not clear whether the number of cell-surface receptors was increased since (^{3}H)diprenorphine binding was employed to quantitate both intracellular and extracellular receptors. Recently, Chaipatikul *et al.* (34) demonstrated that two deletion mutants of rMOPRs which were poorly expressed on the plasma membrane were mainly localized in the calnexin-enriched intracellular compartment, namely, ER. Cell-surface expression of these two rMOPR mutants was rescued by prolonged treatment with hydrophobic MOPR agonists and antagonists, but not with the inactive isomers and hydrophilic ligands. Moreover, for the C-2 mutants lacking the ^{344}KRCFR^{348} motif preceding the putative palmitoylation site in the carboxyl tail, the rescued receptor

was found to be functional on the cell surface in mediating DAMGO-mediated inhibition of forskolin-stimulated adenylyl cyclase activation. Their study also demonstrated that naloxone-promoted enhancement in cell surface expression of the two rMOPR mutants was completely abolished by brefeldin A and monensin, which block protein transport from ER to cis-Golgi apparatus. Thus, hydrophobic ligands of MOPRs appear to act as pharmacological chaperones as well.

When expressed in CHO cells, the wild-type human kappa opioid receptors (hKOPRs) migrated as two different species: immature (precursor, glycosylated intermediate) and mature (fully glycosylated) forms (35). Recently our group demonstrated that the cell-permeant antagonist naloxone promoted transformation of immature precursor into mature receptor and up-regulated both total and surface (36). In addition, following treatment with brefeldin A, which disrupts Golgi apparatus, naloxone lost its up-regulatory effects on the receptors, indicating that it acts on the ER to facilitate trafficking to cell surface. Possible contributions from regulated DNA transcription, mRNA translation and stability of mature receptors were all excluded. Furthermore, all the other hydrophobic (non-peptide) ligands used in the study, including agonists and antagonists, had similar impacts on ER export and intracellular trafficking of hKOPRs as naloxone. The agonists include U50,488H, TRK-820, ethylketocyclazocine, bremazocine, asimadoline, and ICI 204,448, pentazocine and etorphine. The antagonist norbinaltorphimine also significantly increased the mature receptor. Thus, all of cell-permeant ligands function as pharmacological chaperones to enhance cell surface expression of the hKOPR. In contrast, peptide ligands (dynorphins A and B) had no chaperone-like effects.

Following treatment with brefeldin A, hydrophobic agonists caused higher extent of receptor down-regulation and hydrophobic antagonists lost their up-regulatory effects on the hKOPRs. Thus, chronic ligand treatment potentially has dual effects on receptor levels: activation-induced receptor degradation and chaperone-mediated enhancement. At two ends of the spectrum are the peptide agonists and non-peptide antagonists. Membrane-impermeant peptide agonists cause activation-induced receptor degradation without any chaperone effect. Membrane-permeant antagonists exert chaperone-like actions without activation-promoted degradation. Between the two ends, cell-permeant non-peptide agonists have both effects and their net effects are the algebraic sum of the two. Thus, all non-peptide full agonists lead to smaller extent of down-regulation of the mature receptor than peptide full agonists. For some agonists such as etorphine and pentazocine, which do not induce significant hKOPR internalization, their chaperone-induced up-regulatory effects were more pronounced than other agonists. However, for agonists such as U50,488H, TRK820, asimadoline, of which chaperone-like function is weaker than their down-regulatory function, receptor down-regulation were observed.

Recently, Wannemacher *et al.* (37) reported that a 24-h treatment with etorphine and cyclazocine significantly promoted maturation of rat KOPRs (rKOPRs), but treatment with U69,593 and salvinorin A did not, although all four agonists are cell-permeant and rKOPRs are not internalized and degraded following receptor activation. Thus, cell-permeant ligands showed differential abilities to act as pharmacological chaperones. However, in the study, the possibility of low working concentrations (1 microM) of ligands that might contribute to their lack of chaperone function was not excluded. In accordance with our findings, they demonstrated that ligands up-regulated receptor maturation post-translationally because ligand treatment did not change rKOPR mRNA level and translation efficiency. Moreover, cycloheximide pretreatment, blocking new receptor protein biosynthesis, did not affect the rKOPR up-regulation. Taken together, these results indicate that ligands may increase rKOPR expression on the plasma membrane via functioning as pharmacological chaperones. Interestingly, the study also demonstrated that up-regulated part of the receptors were functional as ligand-promoted rKOPR up-regulation was associated with an enhancement in the E_{max} of agonist-stimulated [^{35}S]GTPgammaS binding.

Taken together, the *in vitro* evidence demonstrates that membrane-permeant opioid ligands function at ER as pharmacological chaperones to facilitate receptor trafficking, maturation and cell- surface expression.

7. MOLECULAR MECHANISMS OF PHARMACOLOGICAL CHAPERONES

Mechanisms underlying actions mediated by these chaperone-like hydrophobic compounds have been explored. Petaja-Repo *et al.* (23) suggested that, due to the direct interactions between ligands and amino acid residues in the seven transmembrane helices of their cognate receptors, these cell-permeant compounds cause formation of additional conformational constraints within the binding pockets that provide extra intramolecular packing force and thus enhance thermostability of the native conformers. Our observation that specific receptor binding is required for the up-regulatory effect of the cell-permeant KOPR ligands is consistent with this notion (36). In addition, mutants of the human melanin-concentrating hormone and vasopressin V_2 receptors, in which the mutated residues are located in the TM helices and involved in the intramolecular interactions, are retained in the ER, and hydrophobic ligands successfully rescue cell surface expression of these mutants (29,38). The findings also strongly support this hypothesis.

Leskela *et al.* (39) demonstrated recently that treatment with ligands significantly increased the heat stability of ER-retained wild-type DOPR and enhanced dissociation of receptor precursors from the molecular chaperone calnexin. In addition, Noorwez *et al.* (16) showed that the ligand 11-cis-7-ring retinal, which quantitatively induces *in vivo* folding, maturation and cell surface expression of P23H-opsin mutant, enhanced chemical and thermal stability of the mutant. These

observations are in line with the notion that pharmacological chaperones increase stability of native conformers and concomitantly enhance differences of free energy (ΔG) between unfolded/incompletely-folded intermediates and properly-folded conformers. More receptors with export competency will be generated in the ER with enhanced stability.

Evidence from different groups indicates that immature forms of 7TMRs directly interact with ER-resided molecular chaperone calnexin (39-41) and that receptor mutants are associated with calnexin for a longer time than the wild-type receptors (40,41). In addition, incubation with hydrophobic antagonists was found to accelerate dissociation of the ER form of the receptors from calnexin (39,40). The increased rate of calnexin dissociation and up-regulation of cell surface receptors in the presence of ligands imply that ER folding rate is increased, which subsequently accelerates ER export.

Binding with the hydrophobic ligands probably stabilizes not only the receptors with native conformations but more importantly those at their transition states between unfolded/incompletely-folded and natively-folded states. By doing so, the ligands decrease the activation energy (E_a) that is the energetic barrier for unfolded /incompletely-folded intermediates to overcome. Hence, it can be hypothesized that folding process becomes easier and folding rate is increased, leading to a chain of accelerated intracellular events along the biosynthetic pathway including calnexin dissociation, ER export and generation of mature receptor. Ultimately, receptor expression on the plasma membrane is enhanced. Our findings (36) that both KOPR maturation rate and maturation extent were increased following naloxone treatment are in agreement with this interpretation.

As a consequence of the pharmacological chaperone actions, there should be a decrease in the proportion of ER-retained receptors being targeted to the proteasome-dependent degradation pathway. Indeed, the observation that treatment with hydrophobic ligands reduces polyubiquitination of human wild-type and mutated vasopressin V_3 receptor (40) is in accord with this inference.

Therefore, the membrane-permeant ligands for OPRs most likely act on the ER as pharmacological chaperones to bind to the newly synthesized receptors, like those for other 7TMRs. The interactions of these ligands with residues within the transmembrane hydrophobic core likely stabilize energy-favorable conformers and their transition states, leading to increased stability, accelerated folding / export or both. Following ligand treatment, the steady-state (apparent) levels of ER-resided immature receptor depend on which mechanism dominates. If increased stability predominates, an increased steady-state immature form will be observed. If accelerated folding/export prevails over enhanced receptor stability, a reduced level will be detected. In addition, the level of ER-resided receptors will not be altered by a ligand that does not function as a pharmacological chaperone. Indeed, our observations that U50,488H, naloxone and dynorphin treatment enhanced, decreased, and had no effect on, respectively, the level of the immature precursor (data not shown) are in line with these inferences.

8. CHRONIC ANTAGONIST-PROMOTED OPR UP-REGULATION *IN VIVO*

In vivo up-regulation of OPRs following chronic antagonist has been observed, most likely due to chaperone-like effects of the antagonists. In 1978, Lahti and Collins (42) found that a 4-week infusion of naloxone caused an increase in opioid binding sites by 40% in rat brain homogenate, but no apparent change in dissociation constant of (^{3}H)naloxone. Subsequently, the receptor selectivity and brain region-specificity of the naloxone/naltrexone-evoked up-regulation of OPRs in rats or mice were determined by many laboratories using immunohistochemistry, receptor autoradiography or radioligand binding (43-46). In most studies, all three major types (mu, delta and kappa) of OPRs were reported to be up-regulated after chronic antagonist exposure.

Similar to the findings from *in vitro* studies, the increased portion of endogenous OPRs were found to be functionally normal *in vivo*. In fact, prior to the first report on chronic naloxone-induced up-regulation of opioid binding sites, Tang and Collins (47) demonstrated that chronic naloxone administration enhanced antinociceptive effects of morphine. Subsequently, many other groups also found that up-regulation of OPRs *in vivo* were accompanied by increased analgesic effects of many opioids, including morphine, methadone, etorphine, oxycodone and U50,488H (48-50), and a potentiation of morphine effects on brain monoamine synthesis and metabolism (51). These results suggest that an enhanced functional capacity occurs as a consequence of the up-regulation of OPRs.

Possible mechanisms that account for the increase of endogenous OPRs were investigated. Unterwald *et al.* (52) demonstrated that increases in MOPR binding after chronic naltrexone administration were not accompanied by an enhancement in MOPR mRNA levels. Tempel *et al.* (53) found that treatment with cycloheximide that blocks protein synthesis did not affect up-regulation of endogenous MOPRs by 7-day naloxone treatment in mouse spinal cord-ganglion explants. Taken together, these findings indicate that the antagonists do not affect DNA transcription, mRNA translation or mRNA stability and that the antagonists regulate MOPR post-translationally.

Since pharmacological chaperone effects of ligands on both wild-type and mutated OPRs have been clearly established *in vitro*, it is reasonable to extrapolate that the hydrophobic ligands function *in vivo* like molecular chaperones to promote folding and to facilitate ER export of the endogenous OPRs.

Roles of the proteins involved in OPR trafficking in antagonist-induced up-regulation have been explored. Yoburn's group (54-56) showed that chronic treatment with

either naloxone or naltrexone decreased the levels of GRK2 and dynamin 2, suggesting that diminished constitutive internalization of OPRs may contribute to the receptor up-regulation. In contrast, Diaz *et al.* (57) reported that both antagonists significantly enhanced expression levels of GRK2, GRK3, GRK6 and beta-arrestin 2 after 7-day infusion. Therefore, contribution of reduced endocytosis to the receptor up-regulation is still inconclusive.

How long-term treatment with opioid agonists, particularly morphine, regulates the expression of OPRs in animals has been studied. Chronic treatment with morphine has been found to result in no change (58,59), a decrease (60-62) or an increase (63,64) in MOPR expression, The discrepant results probably are caused by different experimental design, brain regions examined and approaches employed for quantitation among the laboratories. In addition, agonists affect not only the protein biosynthesis pathway but also post-activation endocytosis pathway, which may complicate our evaluation of whether they act like molecular chaperones to increase receptor expression.

9. PERSPECTIVES

To date, the pharmacological chaperone-mediated rescue or enhancement of maturation and cell surface expression has been observed for OPRs, both the wild-types and mutants, similar to several other 7TMRs. Deficiencies in cell surface expression of receptors are causes of some diseases, for examples nephrogenic diabetes insipidus, hypogonadotropic hypogonadism and retinitis pigmentosa, and their chaperone-like hydrophobic ligands appear to be therapeutically useful (for a review, see (31)). Currently, there is no direct evidence linking low cell surface expression of wild-type or mutated OPRs due to misfolding to any disease, but it is desirable to consider the roles of receptor up-regulation mediated by cell-permeant opioids in their chronic pathophysiological or pharmacological functions because long-term administration of either opioid agonists or opioid antagonists is quite common in patients and drug abusers. In addition, immunoelectron microscopy showed that while most of the MOPRs in the rat brain neurons were present on cell surface (65,66), the majorities of rat DOPRs and KOPRs were localized intracellularly not associated with any organelles (66-69). Whether pharmacological chaperones affect their subcellular distributions remains to be investigated. Since some full or partial agonists have little propensity to internalize the activated OPRs, it seems to be valuable to study their clinical implications. Furthermore, our current understanding indicates that non-peptide ligands have an added advantage over peptides in that they can act as pharmacological chaperones. Thus, non-peptide agonists will cause less down-regulation of the receptors than peptide agonists, leading to less tachyphylaxis following long-term treatment.

10. ACKNOWLEDGMENT

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11. REFERENCES

1. Y. Law, Y. H. Wong and H. H. Loh: Molecular mechanisms and regulation of opioid receptor signaling. *Ann Rev Pharmacol Toxicol* 40, 389-430 (2000)

2. M. von Zastrow, A. Svingos, H. Haberstock-Debic and C. Evans: Regulated endocytosis of opioid receptors: cellular mechanisms and proposed roles in physiological adaptation to opiate drugs. *Curr Opin Neurobiol* 13, 348-353 (2003)

3. B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. Molecular Biology of the Cell. Garland Science, New York. (2002)

4. M. von Zastrow: Mechanisms regulating membrane trafficking of G protein-coupled receptors in the endocytic pathway. *Life Sci* 74, 217-224 (2003)

5. M. Waldhoer, S. E. Bartlett and J. L. Whistler: Opioid receptors. *Annu Rev Biochem* 73, 953-990 (2004)

6. L. Ellgaard and A. Helenius: Quality control in the endoplasmic reticulum. *Nat Rev Mol Cell Biol* 4, 181-191 (2003)

7. M. P. Krebs, S. M. Noorwez, R. Malhotra and S. Kaushal: Quality control of integral membrane proteins. *Trends Biochem Sci* 29, 648-655 (2004)

8. B. Kleizen and I. Braakman: Protein folding and quality control in the endoplasmic reticulum. *Curr Opin Cell Biol* 16, 343-349 (2004)

9. A. Kuryatov, J. Luo, J. Cooper and J. Lindstrom: Nicotine acts as a pharmacological chaperone to up-regulate human alpha4beta2 acetylcholine receptors. *Mol Pharmacol* 68, 1839-1851 (2005)

10. E. Ficker, C. A. Obejero-Paz, S. Zhao and A. M. Brown: The binding site for channel blockers that rescue misprocessed human long QT syndrome type 2 ether-a-gogo-related gene (HERG) mutations. *J Biol Chem* 277, 4989-4998 (2002)

11. M. S. Gelman and R. R. Kopito: Rescuing protein conformation: prospects for pharmacological therapy in cystic fibrosis. *J Clin Invest* 110, 1591-1597 (2002)

12. C. Castro-Fernandez, G. Maya-Nunez and P. M. Conn: Beyond the signal sequence: protein routing in health and disease. *Endocr Rev* 26, 479-503 (2005)

13. V. Bernier, D. G. Bichet and M. Bouvier: Pharmacological chaperone action on G-protein-coupled receptors. *Curr Opin Pharmacol* 4, 528-533 (2004)

14. J. P. Morello, A. Salahpour, A. Laperriere, V. Bernier, M. F. Arthus, M. Lonergan, U. Petaja-Repo, S. Angers, D. Morin, D. G. Bichet and M. Bouvier: Pharmacological chaperones rescue cell-surface expression and function of

misfolded V2 vasopressin receptor mutants. *J Clin Invest* 105, 887-895 (2000)

15. J. A. Janovick, G. Maya-Nunez and P. M. Conn: Rescue of hypogonadotropic hypogonadism-causing and manufactured GnRH receptor mutants by a specific protein-folding template: misrouted proteins as a novel disease etiology and therapeutic target. *J Clin Endocrinol Metab* 87, 3255-3262 (2002)

16. S. M. Noorwez, V. Kuksa, Y. Imanishi, L. Zhu, S. Filipek, K. Palczewski and S. Kaushal: Pharmacological chaperone-mediated *in vivo* folding and stabilization of the P23H-opsin mutant associated with autosomal dominant retinitis pigmentosa. *J Biol Chem* 278, 14442-14450 (2003)

17. K. Romisch: A cure for traffic jams: small molecule chaperones in the endoplasmic reticulum. *Traffic* 5, 815-820 (2004)

18. D. H. Perlmutter: Chemical chaperones: a pharmacological strategy for disorders of protein folding and trafficking. *Pediatr Res* 52, 832-836 (2002)

19. A. Ulloa-Aguirre, J. A. Janovick, S. P. Brothers and P. M. Conn: Pharmacologic rescue of conformationally-defective proteins: implications for the treatment of human disease. *Traffic* 5, 821-837 (2004)

20. V. Bernier, J. P. Morello, A. Zarruk, N. Debrand, A. Salahpour, M. Lonergan, M. F. Arthus, A. Laperriere, R. Brouard, M. Bouvier and D. G. Bichet: Pharmacologic chaperones as a potential treatment for X-linked nephrogenic diabetes insipidus. *J Am Soc Nephrol* 17, 232-243 (2006)

21. S. Ishii, H. Yoshioka, K. Mannen, A. B. Kulkarni and J. Q. Fan: Transgenic mouse expressing human mutant alpha-galactosidase A in an endogenous enzyme deficient background: a biochemical animal model for studying active-site specific chaperone therapy for Fabry disease. *Biochim Biophys Acta* 1690, 250-257 (2004)

22. V. Bernier, M. Lagace, D. G. Bichet and M. Bouvier: Pharmacological chaperones: potential treatment for conformational diseases. *Trends Endocrinol Metab* 15, 222-228 (2004)

23. U. E. Petaja-Repo, M. Hogue, S. Bhalla, A. Laperriere, J. P. Morello and M. Bouvier: Ligands act as pharmacological chaperones and increase the efficiency of delta opioid receptor maturation. *EMBO J* 21, 1628-1637 (2002)

24. U. Schubert, L. C. Anton, J. Gibbs, C. C. Norbury, J. W. Yewdell and J. R. Bennink: Rapid degradation of a large fraction of newly synthesized proteins by proteasomes. *Nature* 404, 770-774 (2000)

25. P. Samama, R. A. Bond, H. A. Rockman, C. A. Milano and R. J. Lefkowitz: Ligand-induced overexpression of a constitutively active beta2-adrenergic receptor: pharmacological creation of a phenotype in transgenic mice. *Proc Natl Acad Sci U S A* 94, 137-141 (1997)

26. E. J. Nestler: Molecular basis of long-term plasticity underlying addiction. *Nat Rev Neurosci* 2, 119-128 (2001)

27. P. M. Conn, P. E. Knollman, S. P. Brothers and J. A. Janovick: Protein folding as posttranslational regulation: evolution of a mechanism for controlled plasma membrane expression of a G protein-coupled receptor. *Mol Endocrinol* 20, 3035-3041 (2006)

28. K. Van Craenenbroeck, S. D. Clark, M. J. Cox, J. N. Oak, F. Liu and H. H. Van Tol: Folding efficiency is rate-limiting in dopamine D4 receptor biogenesis. *J Biol Chem* 280, 19350-19357 (2005)

29. S. Wuller, B. Wiesner, A. Loffler, J. Furkert, G. Krause, R. Hermosilla, M. Schaefer, R. Schulein, W. Rosenthal and A. Oksche: Pharmacochaperones post-translationally enhance cell surface expression by increasing conformational stability of wild-type and mutant vasopressin V2 receptors. *J Biol Chem* 279, 47254-47263 (2004)

30. J. P. Fortin, E. K. Dziadulewicz, L. Gera and F. Marceau: A nonpeptide antagonist reveals a highly glycosylated state of the rabbit kinin B1 receptor. *Mol Pharmacol* 69, 1146-1157 (2006)

31. P. M. Conn, A. Ulloa-Aguirre, J. Ito and J. A. Janovick: G protein-coupled receptor trafficking in health and disease: lessons learned to prepare for therapeutic mutant rescue *in vivo*. *Pharmacol Rev* 59, 225-250 (2007)

32. U. E. Petaja-Repo, M. Hogue, A. Laperriere, P. Walker and M. Bouvier: Export from the endoplasmic reticulum represents the limiting step in the maturation and cell surface expression of the human delta opioid receptor. *J Biol Chem* 275, 13727-13736 (2000)

33. J. Li, C. Chen, P. Huang and L.-Y. Liu-Chen: Inverse agonist up-regulates the constitutively active D3.49 (164)Q mutant of the rat μ opioid receptor by stabilizing the structure and blocking constitutive internalization and down-regulation. *Mol Pharmacol* 60, 1064-1075 (2001)

34. V. Chaipatikul, L. J. Erickson-Herbrandson, H. H. Loh and P. Y. Law: Rescuing the traffic-deficient mutants of rat mu-opioid receptors with hydrophobic ligands. *Mol Pharmacol* 64, 32-41 (2003)

35. C. Chen, J. G. Li, Y. Chen, P. Huang, Y. Wang and L. Y. Liu-Chen: GEC1 interacts with the kappa opioid receptor and enhances expression of the receptor. *J Biol Chem* 281, 7983-7993 (2006)

36. Y. Chen, C. Chen, Y. Wang and L. Y. Liu-Chen: Ligands regulate cell surface level of the human {kappa} opioid receptor by activation-induced down-regulation and pharmacological chaperone-mediated enhancement:

Differential effects of nonpeptide and peptide agonists. *J Pharmacol Exp Ther* 319, 765-775 (2006)

37. K. M. Wannemacher, P. N. Yadav and R. D. Howells: A select set of opioid ligands induce up-regulation by promoting the maturation and stability of the rat kappa-opioid receptor in human embryonic kidney 293 cells. *J Pharmacol Exp Ther* 323, 614-625 (2007)

38. J. Fan, S. J. Perry, Y. Gao, D. A. Schwarz and R. A. Maki: A point mutation in the human melanin concentrating hormone receptor 1 reveals an important domain for cellular trafficking. *Mol Endocrinol* 19, 2579-2590 (2005)

39. T. T. Leskela, P. M. Markkanen, E. M. Pietila, J. T. Tuusa and U. E. Petaja-Repo: Opioid receptor pharmacological chaperones act by binding and stabilizing newly synthesized receptors in the endoplasmic reticulum. *J Biol Chem* (2007)

40. J. Robert, C. Auzan, M. A. Ventura and E. Clauser: Mechanisms of cell-surface rerouting of an endoplasmic reticulum-retained mutant of the vasopressin V1b/V3 receptor by a pharmacological chaperone. *J Biol Chem* 280, 42198-42206 (2005)

41. J. P. Morello, A. Salahpour, U. E. Petaja-Repo, A. Laperriere, M. Lonergan, M. F. Arthus, I. R. Nabi, D. G. Bichet and M. Bouvier: Association of calnexin with wild type and mutant AVPR2 that causes nephrogenic diabetes insipidus. *Biochem* 40, 6766-6775 (2001)

42. R. A. Lahti and R. J. Collins: Chronic naloxone results in prolonged increases in opiate binding sites in brain. *Eur J Pharmacol* 51, 185-186 (1978)

43. B. J. Morris, M. J. Millan and A. Herz: Antagonist-induced opioid receptor up-regulation. II. Regionally specific modulation of *mu*, *delta* and *kappa* binding sites in rat brain revealed by quantitative autoradiography. *J Pharmacol Exp Ther* 247, 729-736 (1988)

44. A. L. Giordano, B. Nock and T. J. Cicero: Antagonist-induced up-regulation of the putative *epsilon* opioid receptor in rat brain: Comparison with *kappa, mu* and *delta* opioid receptors. *J Pharmacol Exp Ther* 255, 536-540 (1990)

45. C. M. Paden, S. Krall and W. C. Lynch: Heterogeneous distribution and upregulation of mu, delta and kappa opioid receptors in the amygdala. *Brain Res* 418, 349-355 (1987)

46. H. M. Lesscher, A. Bailey, J. P. Burbach, J. M. van Ree, I. Kitchen and M. A. Gerrits: Receptor-selective changes in mu-, delta- and kappa-opioid receptors after chronic naltrexone treatment in mice. *Eur J Neurosci* 17, 1006-1012 (2003)

47. A. H. Tang and R. J. Collins: Enhanced analgesic effects of morphine after chronic administration of naloxone in the rat. *Eur J Pharmacol* 47, 473-474 (1978)

48. M. J. Millan, B. J. Morris and A. Herz: Antagonist-induced opioid receptor up-regulation. I. Characterization of supersensitivity to selective *Mu* and *Kappa* agonists. *J Pharmacol Exp Ther* 247, 721-728 (1988)

49. B. C. Yoburn, S. Shah, K. Chan, A. Duttaroy and T. Davis: Supersensitivity to opioid analgesics following chronic opioid antagonist treatment: relationship to receptor selectivity. *Pharmacol Biochem Behav* 51, 535-539 (1995)

50. A. Tempel, E. L. Gardner and R. S. Zukin: Neurochemical and functional correlates of naltrexone-induced opiate receptor up-regulation. *J Pharmacol Exp Ther* 232, 439-444 (1985)

51. L. Ahtee, L. M. Attila and K. R. Carlson: Augmentation of morphine-induced changes in brain monoamine metabolism after chronic naltrexone treatment. *J Pharmacol Exp Ther* 255, 803-808 (1990)

52. E. M. Unterwald, J. M. Rubenfeld, Y. Imai, J. B. Wang, G. R. Uhl and M. J. Kreek: Chronic opioid antagonist administration upregulates mu opioid receptor binding without altering mu opioid receptor mRNA levels. *Brain Res* Molecular Brain Research. 33, 351-355 (1995)

53. A. Tempel, S. M. Crain, E. R. Peterson, E. J. Simon and R. S. Zukin: Antagonist-induced opiate receptor upregulation in cultures of fetal mouse spinal cord-ganglion explants. *Brain Res* 390, 287-291 (1986)

54. V. Rajashekara, C. N. Patel, K. Patel, V. Purohit and B. C. Yoburn: Chronic opioid antagonist treatment dose-dependently regulates mu-opioid receptors and trafficking proteins *in vivo*. *Pharmacol Biochem Behav* 75, 909-913 (2003)

55. B. C. Yoburn, V. Purohit, K. Patel and Q. Zhang: Opioid agonist and antagonist treatment differentially regulates immunoreactive mu-opioid receptors and dynamin-2 *in vivo*. *Eur J Pharmacol* 498, 87-96 (2004)

56. C. N. Patel, V. Rajashekara, K. Patel, V. Purohit and B. C. Yoburn: Chronic opioid antagonist treatment selectively regulates trafficking and signaling proteins in mouse spinal cord. *Synapse* 50, 67-76 (2003)

57. A. Diaz, A. Pazos, J. Florez, F. J. Ayesta, V. Santana and M. A. Hurle: Regulation of mu-opioid receptors, G-protein-coupled receptor kinases and beta-arrestin 2 in the rat brain after chronic opioid receptor antagonism. *Neuroscience* 112, 345-353 (2002)

58. B. C. Yoburn, V. Sierra and K. Lutfy: Simultaneous development of opioid tolerance and opioid antagonist-

induced receptor upregulation. *Brain Res* 529, 143-148 (1990)

59. M. B. Patel, C. N. Patel, V. Rajashekara and B. C. Yoburn: Opioid agonists differentially regulate mu-opioid receptors and trafficking proteins *in vivo*. *Mol Pharmacol* 62, 1464-1470 (2002)

60. H. N. Bhargava and A. Gulati: Down-regulation of brain and spinal cord μ-opiate receptors in morphine tolerant-dependent rats. *Eur J Pharmacol* 190, 305-311 (1990)

61. K. Nishino, Y. F. Su, C.-S. Wong, W. D. Watkins and K.-J. Chang: Dissociation of mu opioid tolerance from receptor down-regulation in rat spinal cord. *J Pharmacol Exp Ther* 253, 67-72 (1990)

62. A. Tempel: Visualization of μ opiate receptor downregulation following morphine treatment in neonatal rat brain. *Dev Brain Res* 64, 19-26 (1991)

63. L. S. Brady, M. Herkenham, J. B. Long and R. B. Rothman: Chronic morphine increases mu-opiate receptor binding in rat brain: a quantitative autoradiographic study. *Brain Res* 477, 382-386 (1989)

64. R. B. Rothman, V. Bykov, J. B. Long, L. S. Brady, A. E. Jacobson, K. C. Rice and J. W. Holaday: Chronic administration of morphine and naltrexone up-regulate mu-opioid binding sites labeled by (3H) (D-Ala2,MePhe4,Gly-ol5)enkephalin: further evidence for two mu-binding sites. *Eur J Pharmacol* 160, 71-82 (1989)

65. E. J. Van Bockstaele and K. G. Commons: Internalization of mu-opioid receptors produced by etorphine in the rat locus coeruleus. *Neuroscience* 108, 467-477 (2001)

66. H. Wang and V. M. Pickel: Preferential cytoplasmic localization of delta-opioid receptors in rat striatal patches: comparison with plasmalemmal mu-opioid receptors. *J Neurosci* 21, 3242-3250 (2001)

67. C. M. Cahill, K. A. McClellan, A. Morinville, C. Hoffert, D. Hubatsch, D. O'Donnell and A. Beaudet: Immunohistochemical distribution of delta opioid receptors in the rat central nervous system: evidence for somatodendritic labeling and antigen-specific cellular compartmentalization. *J Comp Neurol* 440, 65-84 (2001)

68. J. A. Harris, P. C. Chang and C. T. Drake: Kappa opioid receptors in rat spinal cord: sex-linked distribution differences. *Neuroscience* 124, 879-890 (2004)

69. S. J. Shuster, M. Riedl, X. Li, L. Vulchanova and R. Elde: Stimulus-dependent translocation of kappa opioid receptors to the plasma membrane. *J Neurosci* 19, 2658-2664 (1999)

Abbreviations: 7TMRs: seven transmembrane receptors, ER: endoplasmic reticulum, ERAD: endoplasmic reticulum-associated degradation, ERGIC: endoplasmic reticulum-Golgi intermediate compartment, GnRHR: gonadotropin-releasing hormone receptor, GRKs: G protein-coupled receptor kinases, hDOPRs: human delta opioid receptors, hKOPRs: human kappa opioid receptors, OPRs: opioid receptors, rKOPRs: rat kappa opioid receptors, rMOPRs: rat mu opioid receptors, TGN: trans-Golgi network

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