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# COMMENTARY

# Role of protein kinase C in functional selectivity for desensitization at the μ-opioid receptor: from pharmacological curiosity to therapeutic potential

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Opioid agonists are the best therapy for moderate to severe pain, but clinical use is limited due to the development of tolerance and dependence. For the first time, Bailey and co-workers have demonstrated functional selectivity for agonist-induced desensitization of μ-opioid receptors (MOR) in mature rat locus coeruleus neurons. Native MORs are differentially desensitized through separate, agonist-dependent signalling pathways; desensitization of the morphine-occupied receptor occurs via a protein kinase C alpha-dependent pathway while [D-Ala$^2$, N-MePhe$^4$, Gly-ol]enkephalin-mediated desensitization is via a G protein receptor kinase subtype 2-dependent mechanism. These results suggest that MORs adopt separate conformational states that either result in different efficiencies of G protein activation or access to phosphorylation by desensitization machinery (e.g. protein kinase C alpha or G protein receptor kinase subtype 2). Further study of the interaction of protein kinase C with MORs in native neurons will enhance our understanding of agonist-induced functional selectivity for desensitization at MORs and provide important insights into how to selectively modulate agonist efficacy to enhance therapeutic capabilities of opioid drugs.
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**Keywords:** morphine; μ-opioid receptor; PKC; opioid; opiate; desensitization; tolerance; G protein-coupled receptor kinase (GRK); DAMGO; G protein

**Abbreviations:** DAMGO, [D-Ala$^2$, N-MePhe$^4$, Gly-ol]enkephalin; GIRK, G protein coupled inwardly rectifying potassium channel; GPCR, G protein-coupled receptor; GRK2, G protein receptor kinase subtype 2; LC, locus coeruleus; MOR, μ-opioid receptor; PKCα, protein kinase C alpha

Agonists of the μ-opioid receptor (MOR) are the most appropriate therapy for moderate to severe pain, although issues of the development of tolerance and dependence diminish their usefulness in patients requiring chronic treatment. Much work has been done to elucidate the cellular mechanisms of opioid-induced analgesia, tolerance and dependence in the hope of finding an analgesic that is capable of enhancing pain relief without initiating the cellular processes involved in tolerance and dependence. Current work on G protein-coupled receptors (GPCRs), of which opioid receptors are one family, have demonstrated that different agonists are capable of 'functional selectivity', that is, initiating ligand-dependent conformational states that result in differential signalling mechanisms (Urban *et al.*, 2007). The data presented in this issue of the *British Journal of Pharmacology* by Bailey *et al.* (2009) provide evidence for functional selectivity of agonist-induced desensitization of MORs in mature rat locus coeruleus (LC) neurons. This work is particularly significant in that, for the first time, native MORs in mature neurons are shown to be differentially desensitized through separate, agonist-dependent signalling pathways.

Bailey *et al.* (2009) show that acute morphine-induced desensitization is mediated through protein kinase C (PKC)α in mature LC neurons while [D-Ala$^2$, N-MePhe$^4$, Gly-ol]enkephalin (DAMGO)-induced desensitization occurs via the more traditional and accepted G protein receptor kinase

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(GRK) subtype 2 (GRK2)-dependent mechanism. GRK2 is recruited to the membrane by Gβγ subunits released following activation of heterotrimeric G proteins by agonist-occupied receptors. Both morphine and DAMGO cause activation of G proteins and show no marked differences in the types of Gα proteins with which they interact (Clark *et al.*, 2006). They also exhibit the same qualitative signalling responses, although the potency and efficacy for DAMGO is generally higher. This may be important because activation of Gβγ is a very early event following receptor occupancy and so requires considerably more agonist efficacy than events further downstream. Morphine is a low-efficacy agonist at the level of G protein and may be insufficiently efficacious to recruit GRK2 to effectively phosphorylate the receptor. The fact that the binding domain on Gβγ for GRK2 overlaps considerably with the binding sites for other Gβγ effectors, including PLCβ and ion channels, so that these proteins compete with GRK2 for binding to Gβγ (Lodowski *et al.*, 2003) emphasizes the low level of activation of Gβγ by morphine. This may explain why increasing the level of GRK2 in a cell does result in GRK2-mediated internalization of MOR (see Connor *et al.*, 2004). Further support for this idea comes from observations that Gβγ does have differential ability to activate effectors as seen, for example, when comparing MOR activation of membrane delimited Gβγ coupling to ion channels in LC neurons. MOR activation of G protein coupled, inwardly rectifying, potassium channels (GIRK) and $Ca^{2+}$ channels, via lateral diffusion of released Gβγ subunits, are the fastest measures of MOR coupling in neurons (Connor *et al.*, 2004). Although the efficacy of morphine to stimulate GIRK and inhibit $Ca^{2+}$ channels is lower than that of DAMGO, morphine is more effective at inhibiting $Ca^{2+}$ channels than activating GIRK channels in isolated LC neurons (Ingram *et al.*, 1997). These results moreover suggest that functional differences in efficacy are mediated, in part, via mechanisms downstream of the MOR, at least with acute administration of agonists. Many studies have demonstrated that significant changes occur in MOR regulation after chronic administration of opioids (see Christie, 2008).

An alternative explanation for the failure of morphine to promote GRK-mediated phosphorylation of MOR is that GRK recruitment by the Gβγ subunits does occur, but that the GRK is unable to phosphorylate the morphine-occupied MOR. Following recruitment to the plasma membrane, GRK2 is oriented to facilitate phosphorylation of activated GPCRs. However, the activated GPCR alters the conformation of the GRK such that the kinase is activated (Singh *et al.*, 2008). Thus, the morphine-occupied MOR may not be phosphorylated because it is not in a conformation, or set of conformations, which allows for activation of the kinase activity of GRK2 and/or for phosphorylation by GRK2.

The hypothesis that agonists acting at the same GPCR show functional selectivity for different signalling pathways, based on agonist-specific receptor conformations and/or differential strength of signalling dependent on agonist efficacy, has become popular over the past decade (see Connor *et al.*, 2004; Urban *et al.*, 2007). The plethora of agonists for, and interest in, signalling pathways of MORs has helped shape the concept of functional selectivity and the prototypical full and partial agonists, DAMGO and morphine, respectively, have been used extensively to explore MOR signalling (see Connor *et al.*, 2004; Christie, 2008). These studies demonstrate that there are differences in agonist profiles for antinociception, GIRK activation, mitogen-activated protein kinase activation, adenylyl cyclase inhibition, desensitization and internalization. However, the case for physiological functional selectivity of agonist-induced MOR signalling has not yet been made and the question remains as to whether differences in agonist-induced MOR signalling are the result of physiologically relevant agonist-specific conformational states of MORs, the particular subset of signalling proteins and their regulators, the presence of the receptor in certain membrane microdomains or a combination of the above. One major difficulty in comparing studies is the conditions under which each study is performed, including heterologous versus endogenous expression, expression levels of receptors and modulators, effector-coupling mechanisms studied and different cell types, species and duration of drug treatments (acute vs. chronic).

The issues associated with defining functional selectivity underscore the need to study receptor interactions in native tissues with endogenous levels of receptors while paying close attention to the temporal resolution of receptor–effector coupling. Bailey *et al.* (2009) demonstrate that morphine-induced desensitization of MORs in mature LC neurons is minimal *in vitro* in the absence of PKC stimulation by either phorbol esters or via concomitant stimulation of PKC by $M_3$ muscarinic receptors. Consequently, it does not appear that morphine binding to the MOR activates PKC activity in LC neurons, although morphine occupancy of MOR is needed for effective desensitization. Therefore, the observed agonist-induced functional selectivity for desensitization does not look to be a result of differential activation of effector systems (GRK vs. PKC), but rather that PKC recognizes a MOR conformation(s) that may be specific to morphine compared with DAMGO occupation of the binding site, although it has not been ruled out that PKCα could play a role in desensitization of the DAMGO-occupied MOR in the absence of GRK2 activity. Certainly in analysing the findings of Bailey *et al.* (2009), it should be noted that the insensitivity of morphine to GRK2 is not absolute. For example, in cultured rat striatal neurons morphine induces rapid endocytosis of MOR (Haberstock-Debic *et al.*, 2005) and in heterologous systems with GRK2 overexpression, morphine alone can cause internalization of MOR following GRK2-mediated phosphorylation (see Connor *et al.*, 2004). Thus, it is likely that the morphine-occupied MOR does directly recruit GRK and undergoes receptor phosphorylation, for example, in cells expressing higher G protein or GRK levels or in cells in which negative regulators of G protein signalling that allow for the rapid recombination of Gα and Gβγ subunits are low or ineffective. Under these conditions, levels of released Gβγ subunits are increased (Clark *et al.*, 2003) and may recruit sufficient GRK2 for phosphorylation of the morphine-occupied MOR.

The data presented by Bailey *et al.* (2009) provide evidence that PKC, activated by a MOR-independent mechanism, may potentially recognize a morphine-specific conformation of MOR and so cause receptor desensitization. What, then, are the implications of this alternative desensitization mechanism? Desensitization of the receptor, followed by

re-sensitization, is vital for the cell to prevent continual bombardment by neurotransmitter signals and be in a position to respond to new incoming signals. Thus, the cell provides more than one pathway to achieve this, including the PKCα-mediated desensitization mechanism, which functions for agonists that do not efficiently initiate homologous, GRK-mediated, desensitization. Further study of the interaction of PKC with MORs in native neurons will enhance our understanding of the complex regulation associated with agonist activation of MORs. Certainly, the *in vivo* observations that PKC inhibitors can reduce morphine anti-nociceptive tolerance (Smith *et al*., 2007) provide evidence that selective inhibition of those PKCs involved, or pharmacological intervention at $G\alpha_q$-linked receptors co-expressed in similar locations to MORs within target neurons, might provide useful adjuncts to chronic therapy with opioid drugs.

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