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# COMMENTARY

# Shadows across μ-Star? Constitutively active μ-opioid receptors revisited

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Constitutively active μ-opioid receptors (μ* receptors) are reported to be formed following prolonged agonist treatment of cells or whole animals. μ* receptors signal in the absence of activating ligand and a blockade of μ* activation of G-proteins by naloxone and naltrexone has been suggested to underlie the profound withdrawal syndrome precipitated by these antagonists *in vivo*. In this issue of the Journal, Divin *et al.* examined whether treatment of C6 glioma cells with μ-opioid receptor agonists produced constitutively active μ-opioid receptors or other commonly reported adaptations to prolonged agonist treatment. Adenylyl cyclase superactivation was readily apparent following agonist treatment but there was no evidence of the formation of constitutively active μ-opioid receptors. This result challenges the notion that prolonged agonist exposure inevitably produces μ* receptors, and is consistent with many studies of adaptations in neurons produced by chronic agonist treatment. The investigators provide no explanation of their failure to see μ* receptors in C6 cells, but this is perhaps understandable because the molecular nature of μ* receptors remains elusive, and the precise mechanisms that lead to their formation are unknown. Without knowing exactly what μ* receptors are, how they are formed and how they signal, understanding their role in cellular adaptations to prolonged opioid treatment will remain impossible. Studies such as this should refocus attention on establishing the molecular mechanisms that underlie that phenomenon of μ* receptors.
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## Main text

G-protein-coupled receptors are still being shown to signal in new and exciting ways but one of the earliest surprises was the realization that the receptors could activate G-proteins in the absence of ligand. Constitutive activity has subsequently been measured for a wide variety of receptors, and this has been facilitated by the identification of many receptor antagonists as 'inverse agonists'; drugs that stabilize conformation(s) of the receptor that do not couple to G-proteins (Kenakin, 2004). While the importance of constitutive receptor activity in physiological processes is still debated, the induction of constitutive activity by drugs has been proposed to underlie two intensively studied pathopharmacological phenomena – opioid tolerance and withdrawal (Sadee *et al.*, 2005).

As originally described, constitutively active μ-opioid receptors are a phosphorylated form of the receptor (μ*) that signals independently of ligand binding (Wang *et al.*, 1994). μ* receptors are formed during prolonged (hours to days) exposure of cells to both peptide and non-peptide μ-opioid receptor agonists, and they reportedly persist for hours or days (Wang *et al.*, 2004) following removal of agonist. Importantly, the activity of μ* receptors is blocked by commonly used opioid antagonists such as naloxone and naltrexone, leading to the appearance of apparent inverse agonist properties of these drugs after chronic agonist treatment. Inverse agonist activity is reflected by a ligand-induced *decrease* in basal G-protein activity and an *increase* in adenylyl cyclase activity resulting from the inverse agonist block of the tonic inhibition of adenylyl cyclase by the $G_i$-coupled μ* receptors. Constitutively active μ-opioid receptors are important because they have been postulated to provide continuous signalling during treatment with opioid agonists that may produce desensitization of conventional μ-opioid receptor/G-protein signalling as well as providing persistent signalling after chronic morphine treatment has been terminated by clearance of agonist or administration of antagonist. μ* receptors also represent an unusual species because their constitutive activity is proposed to be produced by a post-translational modification triggered by agonist exposure, rather than reflecting an intrinsic property of the receptor in a 'basal' state.

In this issue of the Journal, Divin *et al.* (2008a) have examined the induction of constitutive μ-opioid receptor activity

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by chronic treatment of C6 glioma cells with the high efficacy peptide agonist Tyr-D-Ala-Gly[*N*-MePhe]-NH($CH_2$)$_2$ (DAMGO) and the partial agonist alkaloid morphine. They report that while agonist treatment produces the typical sensitization of adenylyl cyclase activity and decrease in agonist-stimulated GTPγS binding, agonist treatment does not result in the appearance of inverse agonist activity for naloxone or naltrexone. In fact, the effects of naloxone and naltrexone on adenylyl cyclase activity and basal GTPγS binding are the same as that of the putative neutral antagonist 6β-naltrexol. Further, the study finds that the small amount of inverse agonist activity observed for the δ-opioid receptor ligand (+)-*N*-[*trans*-4′(2-methylphenyl)-2′-butenyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (RTI-5989-25) disappears after agonist treatment, rather than being increased. Finally, the work shows that the peptide antagonist D-Phe-cyc[Cys-Tyr-D-Trp-Arg-Thr-Pen]-Thr-$NH_2$ (CTAP) displays protean characteristics depending on the assay conditions, further underlying the complexities of ligand/receptor interactions and their subsequent signalling consequences. The most important result of the present study is that it clearly demonstrates that formation of μ* receptors is not an inevitable consequence of prolonged agonist treatment, even if this treatment produces other characteristic cellular adaptations. The most important immediate effect of the work is to highlight how much we do not know about μ* receptors.

Inverse agonist activity of the μ-opioid receptor has been reported by many groups, with a variety of ligands, in several different cells lines *in vitro*, usually after chronic agonist treatment (reviewed in Sadee *et al.*, 2005). Although cell lines differ from each other in many ways, and μ* receptor activity has been measured using different experimental conditions in different labs, a ready explanation of the variant results reported by Divin *et al.* is not apparent because the molecular nature of the μ* state is unknown and the processes that lead to its creation have been described only in the broadest terms. Most importantly, the residue(s) of the μ-opioid receptor that are phosphorylated to produce the μ* state have not been identified and little is known about the protein kinase(s) responsible other than that they are blocked by high concentrations of the protein kinase inhibitor H7. It is also not known if the μ* state is promoted directly as a result of agonist occupancy of the receptor, in a manner analogous to putative G-protein receptor kinase phosphorylation of G-protein-coupled receptors, or whether μ* receptors are formed as a result of signalling processes that occur after receptor activation. We also do not know if the μ* receptor represents a single receptor state or a number of interchanging conformations, or whether it signals to the same sets of effectors as agonist-activated μ-opioid receptors. This lack of fundamental information is a major impediment to defining the role of the μ* state in cellular responses to chronic opioid treatment.

The role of μ* receptors in the complex adaptations that occur during chronic opioid treatment of whole animals is very difficult to assess. While inverse agonist activity has been reported for a number of μ-opioid receptor antagonists in *ex vivo* tissue, the amount of inverse agonist activity is not increased for every ligand following chronic morphine treatment, which is not consistent with an increase in the absolute amount of μ* receptor activity (Wang *et al.*, 2004; Raehal *et al.*, 2005). Some studies have reported changes in brain homogenate adenylyl cyclase activity or basal GTPγS binding, consistent with the formation of μ* receptors following chronic morphine treatment (Wang *et al.*, 2004). Others have reported no increase in basal G-protein activity or any effects of naloxone on this activity, even in regions highly enriched in μ-opioid receptors (Selley *et al.*, 1997; Kirschke *et al.*, 2002). Studies of adaptations to chronic morphine treatment in intact neurons have not found differences between withdrawing cells by washing out morphine or superfusing naloxone (e.g. Ingram *et al.*, 1998), or effects of naloxone superfusion consistent with reversal of μ* receptor signalling (e.g. Christie *et al.*, 1987). In whole animals, the effects of putative inverse and neutral antagonists in precipitating withdrawal have been interpreted by some (Wang *et al.*, 2004; Raehal *et al.*, 2005; Sirohi *et al.*, 2007) but not others (Divin *et al.*, 2008b; Li *et al.*, 2008) as providing evidence to support the existence of μ* receptors.

Differences between cell lines is not news, and simple differences in experimental conditions might contribute to divergent results from different laboratories. Nevertheless, when studying a phenomenon as important as adaptation to morphine administration investigators cannot simply shrug off such differences, and the present results of Divin *et al.* (2008a) will hopefully serve to refocus attention on understanding what the molecular mechanisms underlying the μ* receptor state might be. The contribution of constitutively active μ-opioid receptors to the complex adaptations occurring during chronic opioid treatment can only be established when we know exactly what they are, how they are formed and how they signal.

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## Conflict of interest

None to declare.

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