Commentary

The enigma of morphine tolerance: recent insights

1. Introduction

Pain has been described as a necessary evil. Necessary because it is a protective reflex and evil since lives have been made miserable due to it. Morphine and its related alkaloids are still the most effective analgesics in a physician's armoury for treating pain. However, many physicians hesitate to use opioids because of side effects – the primary reason being the development of tolerance and dependence on opioids. Other, equally detrimental effects are inhibition of gastrointestinal motility and respiratory depression. However, a general consensus among physicians has emerged that opioids – particularly long-acting opioids – should form the mainstay of treatment of chronic pain of both cancerous and non-cancerous etiology (McCarberg and Barkin 2001).

2. Tolerance and dependence

From the time that opioid receptors were demonstrated in the nervous system by Pert and Snyder (1973) opioid research over the last several decades has been directed towards understanding the mechanism of tolerance and dependence. Tolerance has been defined as reduced efficacy of a drug after repeated administration, while physical dependence is revealed by the occurrence of a withdrawl response on discontinuing the drug. In humans, withdrawl responses include fever, sweating, yawning, nausea, insomnia and piloerection. Piloerection causes goose flesh and is the origin of the term "cold turkey" used to describe the effects of opioid withdrawl (Rang *et al* 1999). Behavioural dependence is manifested by a craving for the drug. The underlying mechanisms of tolerance and dependence and their interrelationships are not well understood and the relevant literature contains a number of inconsistencies and contradictions (Fleming and Taylor 1995). However, recent research has been able to structure these phenomena into more clearly separable entities.

3. Mu receptor and analgesia

Morphine binds to the μ opioid receptor on the cell surface membrane to produce analgesia as also tolerance and dependence. The endogenous ligands for the receptor, are however, endomorphins (Zadina *et al* 1997) and enkephalins (Mansour *et al* 1995). Thus, the interesting feature of these receptors is that these are activated by both nonpeptide alkaloids like morphine as also structurally distinct, native peptides (Hughes and Kosterlitz 1977).

Opioid receptors are members of the G protein-coupled receptor (GPCR) family (Chen *et al* 1993). The G proteins, specifically activated by the opioid receptors, are the G_i/G_o subtypes, which in turn, increase potassium and decrease calcium levels within neurons (Law and Loh 1999). Consequently, opioids have an inhibitory effect – both in terms of neuronal excitability and neuro-transmitter release (North 1993).

4. Receptor turnover

In the normal course of agonist binding to a G protein-coupled receptor, initial desensitization occurs by phosphorylation (Freedman and Lefkowitz 1996). Phosphorylation of the receptor by G protein-

coupled receptor kinases or GRKs is sufficient to produce a small degree of desensitization (or loss of function) but substantial desensitization occurs only when a cytoplasmic protein called *b*-arrestin binds to the receptor (Kavoor *et al* 1997). There are at least 6 members of the GRK gene family, whose products phosphorylate serine and threonine residues on the GPCRs carboxyl tail (Freedman and Lefkowitz 1996). Threonine at position 394 of the *m* receptor is the primary recognition site for GRKs (Pak *et al* 1997). All GRKs share a common structural organization with a poorly conserved N-terminal domain of ~ 185 residues, a conserved protein kinase catalytic domain of ~ 270 residues and a variable length C-terminal domain of 105–230 residues (Krupnick and Benovic 1998).

The arrestins bind to phosphorylated G protein-coupled receptors and cause desensitization by uncoupling the signal transduction system. The arrestins are a class of soluble proteins that function in concert with GRKs. Visual arrestin was the first to be discovered (Kuhn *et al* 1984). Later, *b*-arrestin1 was identified, which could regulate signalling by the *b*-adrenergic receptor after phosphorylation (Benovic *et al* 1987). *b*-arrestin2 was cloned from bovine brain and was noted to be ubiquitiously distributed like *b*-arrestin1 (Sterne-Marr *et al* 1993). It appears that many of these kinases and arrestins are not receptor specific, inactivating many hundreds of different G protein receptors (Fain 1999).

Generally, for any G protein-coupled receptor, like the *b*-adrenergic receptor, *b*-arrestin directs the agonist-receptor complex to specific clathrin-coated pits for endocytosis (Ferguson *et al* 1996; Oakley *et al* 1999). However, it differs for the *m* receptor, depending on the agonist used. This has been shown in cultured human embryo kidney (HEK) cells transfected with *m* opioid receptor. Binding of etorphine, a nonselective opioid agonist to the receptor, results in the usual *b*-arrestin directed internalization. However, when morphine binds to the receptor, there is hardly any internalization of the receptor (Arden *et al* 1995). This was thought to be due to the lack of phosphorylation of the morphine-*m* receptor complex and *b*-arrestin binding (Zhang *et al* 1998).

5. Importance of internalization of GPCRs

The internalized receptors, sequestered within intracellular vesicles, dissociate from the agonist. The receptors are dephosphorylated by specific membrane associated phosphatases and returned back to the cell surface – a process known as resensitization. Again, *b*-arrestin plays a central role in this regard (Zhang *et al* 1997). It was thus thought that the absence of resensitization of the *m* receptor, upon morphine binding, might lead to receptor blockade and tolerance.

However, in between, an important finding went unnoticed. As long back as 1994, it was reported that following chronic morphine treatment in rats, there was an increase in G protein-coupled receptor kinase2 (GRK2) and *b*-arrestin levels in the locus coeruleus (Terwilliger *et al* 1994). *m* receptors are known to be expressed in the locus coeruleus (Mansour *et al* 1988). It was also shown by Zhang *et al* (1998) that overexpression of GRK2 leads to increased internalization of the *m* receptors ($51 \pm 3\%$) as compared to controls ($5 \pm 4\%$) on binding to morphine. It therefore appears that both GRK2 and *b*-arrestin may have a role in the desensitization of *m* receptors.

6. Recent insights

A paper by Bohn *et al* (1999) had earlier reported that in mice lacking the *b*-arrestin2 gene ($barr2^{-/-}$), the analgesic effect of morphine was more potent and prolonged as compared to controls. The *b*-arrestin2 knockout mice was generated by inactivating the concerned gene through homologous recombination. The generation of transgenic mice deficient in an identified gene, known as knockout mice, was first reported towards the end of the 1980s (Capecchi 1989). An altered and nonfunctional variant of a target gene is synthesized and transfected into pluripotent embryonic stem cells. Identical sequences shared by the native and foreign genes allow recombination to occur, with a chance for intergration of the nonfunctional transgene into some of the embryonic cells. The recombinant stem cells are injected into a blastocyst which is then implanted into a host mother (Nicholls *et al* 2001). The data, presented in the report demonstrated that *b*-arrestin2 does play a role in the eventual desensitization of the receptor. According to Bohn *et al* (1999), the earlier finding by Zhang *et al* (1998) which showed minimum phosphorylation and *b*-arrestin2 binding to the *m* receptor, may have been due to cultured cells being used in the latter study.

However, Sternini *et al* (1996) reported an *in vivo* study where there was no internalization of the *m* receptor in myenteric neurons of the guinea pig ileum after intraperitoneal administration of morphine. In contrast, etorphine triggered significant internalization. Myenteric neurons naturally express the receptor (Sternini *et al* 1995). A more recent study by Sternini *et al* (2000) also noted failure of *m* receptor endocytosis by morphine in ileal muscle-myenteric plexus preparations. It remains to be seen whether phosphorylation and *b*-arrestin2 binding to the *m* receptor is possible without internalization. Bohn *et al* (1999) also suggested that there could be other genetic variants of the *m* receptor, amenable to phosphorylation and *b*-arrestin binding. It was previously shown that the rat *m* receptor isoform rMOR (rat *m* opioid receptor)1 was more easily desensitized than rMOR1B (Hollt *et al* 1997).

Bohn *et al* (2000) recently reported that mice lacking the *b*-arrestin2 gene (*barr2*$^{-/-}$) as before, did not develop tolerance to both acute and chronic administration of morphine. As expected, the intracellular signal transduction mechanism was preserved in these *b*-arrestin2 knockout mice after morphine administration as was evident from [^{35}S]GTP*g*S binding. [^{35}S]GTP*g*S binding provides a functional measure of the efficacy of the signalling pathway after agonist occupation of *m* receptors (Traynor and Nahorski 1995). Notably, there was significant uncoupling in wild type mice. Uncoupling of the receptor from G proteins leads to disruption of the signal transduction mechanism, typically seen in morphine tolerance (Heyliger *et al* 2000). Morever, no significant differences were noted in the density of *m* receptors in brainstem-membrane preparations after chronic morphine administration, both in the wild type and *b*-arrestin2 gene knockout mice. We also observed no significant difference in *m* receptor density between morphine treated and control mice (Ray and Wadhwa 2001). Perhaps the most important finding by Bonn *et al* (2000) was that both groups of mice developed dependence to morphine as shown by an increase of adenylyl cyclase activity, a biochemical marker of dependence (Nestler and Aghajanian 1997).

The elegant series of experiments by Bonn *et al* (2000) have highlighted the role of *b*-arrestin2 in the mechanism of morphine tolerance and also shown that the molecular mechanisms of tolerance and dependence are different. However, developmental compensations are possible in specific gene knockout mice and it may not provide an answer to all problems which the traditional pharmacological approach has left unresolved (Kitchen 1999). Also some inconsistencies exist between different studies, which have to be addressed. For example, opioid receptor-like 1(ORL1) receptor knockout mice show attenuation of tolerance to morphine (Ueda *et al* 1997). Arden *et al* (1995) had reported a small increase of phosphorylation of the *m* receptor on morphine binding (1·8-fold) and the existence of basal phosphorylation, even in the absence of agonist.

It is possible that the role of *b*-arrestin2 is only part of the story, as earlier studies have shown that there are multiple converging cellular events leading to opiate tolerance (Dewey 2001). Other factors could be alterations in intracellular Ca^{2+} (Smith *et al* 1999), increased activity of anti-opioid peptides like nociceptin (Yuan *et al* 1999), increased glutamate in locus coeruleus (Aghajanian *et al* 1994), changes in NR1 subunit of NMDA receptor (Zhu *et al* 1999) and melatonin (Raghavendra and Kulkarni 1999). The ability to reverse morphine tolerance has important implications, which should lead to a better understanding of the mechanisms involved in its development (Dewey 2001). Further studies in this direction thus have great clinical potential.

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SUBRATA BASU RAY*
SHASHI WADHWA
Department of Anatomy,
All India Institute of Medical Sciences,
New Delhi 110 029, India

**(Email, ccourt@medinst.ernet.in)*