Please cite this article in press as: Vicente-Sanchez A et al. The delta opioid receptor tool box. Neuroscience (2016), http://dx.doi.org/10.1016/j.neuroscience.2016.06.028

*Neuroscience* xxx (2016) xxx–xxx

REVIEW

# THE DELTA OPIOID RECEPTOR TOOL BOX

**ANA VICENTE-SANCHEZ, LAURA SEGURA AND AMYNAH A. PRADHAN** *

*Department of Psychiatry, University of Illinois at Chicago, United States*

**Abstract—In recent years, the delta opioid receptor has attracted increasing interest as a target for the treatment of chronic pain and emotional disorders. Due to their therapeutic potential, numerous tools have been developed to study the delta opioid receptor from both a molecular and a functional perspective. This review summarizes the most commonly available tools, with an emphasis on their use and limitations. Here, we describe (1) the cell-based assays used to study the delta opioid receptor. (2) The features of several delta opioid receptor ligands, including peptide and non-peptide drugs. (3) The existing approaches to detect delta opioid receptors in fixed tissue, and debates that surround these techniques. (4) Behavioral assays used to study the *in vivo* effects of delta opioid receptor agonists; including locomotor stimulation and convulsions that are induced by some ligands, but not others. (5) The characterization of genetically modified mice used specifically to study the delta opioid receptor. Overall, this review aims to provide a guideline for the use of these tools with the final goal of increasing our understanding of delta opioid receptor physiology.**

***This article is part of a Special Issue entitled: Pain.*** 

**Key words: pain, cell lines, mutant mice, G protein-coupled receptor.**

Contents

Introduction 00
How to examine delta opioid receptors in cells 00
NG108-15 cell line 00
F11 00
SK-N-BE cell line 00
SK-N-SH cell line 00
SH-SY5Y cell line 00
What ligands bind to the delta opioid receptor 00
How to detect delta opioid receptors 00
Things to consider when examining the behavioral effects of delta agonists 00
Genetic tools to study the delta opioid receptor 00
Delta opioid receptor-KO$_{exon\ 2}$ 00
Delta opioid receptor-KO$_{exon\ 1}$ 00
DOR-eGFP knockin mice 00
Conditional knockout mouse models 00
DOR$^{CMV}$ cKO 00
DOR$^{Nav1.8}$ cKO 00
DOR$^{Dlx5/6}$ cKO 00
Conclusions 00
Acknowledgments 00
References 00

## INTRODUCTION

Opioids have been used for centuries, during which time they have been alternately hailed as both a panacea of man's ills, and cursed as a scourge of civilization. The vast majority of opioids used clinically (eg. morphine, hydrocodone, oxycodone) or recreationally (eg. heroin) produce their behavioral effects primarily through activation of the mu opioid receptor. Although mu agonists are incredibly potent analgesics, they also produce deleterious adverse effects which severely limit their therapeutic use. Activation of the mu opioid receptor can produce constipation, respiratory depression, and sedation. Furthermore, tolerance and physical dependence is observed after chronic use, and the abuse liability of mu agonists is very high.

Another member of the opioid receptor family – the delta opioid receptor – may offer a potential alternative to mu-based therapies. In acute pain states, activation of the delta opioid receptor is relatively ineffective compared to their mu counterparts (Gallantine and Meert, 2005). However, they are highly effective in chronic pain states (Hurley and Hammond, 2000; Fraser et al., 2000a; Cahill et al., 2003; Nadal et al., 2006; Gaveriaux-Ruff et al., 2008; Pradhan et al., 2009, 2010), and delta agonists were also recently shown to be effective for the treatment of migraine (Pradhan et al., 2014). Further, activation of the delta opioid receptor appears to have low abuse liability, as delta agonists are not self-administered (Negus et al., 1998; Stevenson et al., 2005) nor do they cause physical dependence (Brandt et al., 2001). Interestingly, delta opioid receptors also positively modulate emotional tone. Genetic deletion of either the delta opioid receptor or its endogenous ligand, enkephalin, results in anxiogenic and depressive-like behaviors (Konig et al., 1996; Filliol et al., 2000). In contrast, delta agonists can produce anxiolytic and anti-

*Corresponding author. Address: Department of Psychiatry, UIC, 1601 W Taylor Street (MC 912), Chicago, IL 60612, United States.
E-mail address: apradhan@psych.uic.edu (A. A. Pradhan).

http://dx.doi.org/10.1016/j.neuroscience.2016.06.028
0306-4522/

depressant effects (Saitoh et al., 2004; Perrine et al., 2006). This emotional regulation may be particularly important for the treatment of chronic pain states, as there is a high comorbidity with depression and anxiety (Yalcin and Barrot, 2014). The potential of targeting this receptor has not gone unnoticed, and there are ongoing efforts to develop delta opioid receptor agonists for the treatment of a number of psychiatric and neurological conditions (Pradhan et al., 2011a).

During the past 5 years, there have been many reviews published on the delta opioid receptor, which have highlighted their biological function, therapeutic utility, and signaling mechanisms; and we direct the reader to these excellent resources (Pradhan et al., 2011a; Chu Sin Chung and Kieffer, 2013; van Rijn et al., 2013; Gendron et al., 2014; Charfi et al., 2015; Klenowski et al., 2015). The aim of this review, however, is to provide a more practical guide on how to study the delta opioid receptor. Our intention is to discuss the tools that are available to probe delta opioid receptor signaling, distribution, and function; and to highlight some of the pros and cons associated with each method. We hope that this review will be a useful "how to" guide for those interested in working on the delta opioid receptor.

## How to examine delta opioid receptors in cells

The expression of delta opioid receptors in non-neuronal cell lines has served as a powerful tool to delineate delta opioid receptor trafficking and regulation (Whistler et al., 2002; Puthenveedu et al., 2010; Henry et al., 2011), signaling complexes (Audet et al., 2008, 2012; Charfi et al., 2014), and a multitude of other mechanisms. Delta opioid receptors have been transfected into several non-neuronal cell lines, such as the Chinese Hamster Ovary (CHO), the Human Embryonic Kidney (HEK) 293, and the African Green Monkey Kidney (COS-7) (Chan et al., 2003; Hong et al., 2009; Tudashki et al., 2014; Nagi et al., 2015a). Cells are transfected with cDNA encoding the delta opioid receptor, modified to express a peptide epitope or tag within either the N-terminal extracellular region or the C-terminus. Widely used epitope tags include hemagglutinin (HA), c-*myc*, and FLAG (Qiu et al., 2007; Tudashki et al., 2014; Nagi et al., 2015a). Epitope tagging facilitates the detection of the receptor by high-affinity antibodies, and overcomes many of the difficulties posed by using delta opioid receptor specific antibodies (see section below). In addition, delta opioid receptors can also be modified for FRET/BRET- (fluorescence/bioluminescence resonance energy transfer) based technologies, thus allowing for the direct visualization of specific protein–protein interactions (Audet et al., 2008, 2012; Richard-Lalonde et al., 2013; Charfi et al., 2014; Nagi et al., 2015b; Pradhan et al., 2016).

However, it is important to keep in mind some of the limitations of transfected cell systems. There is the possibility that tagging the receptor may affect receptor function. For example, tags can interfere with post-translational processing (Jiang et al., 2012), oligomerization, ligand binding (N-terminal tags), and receptor regulatory and signaling events (C terminus). In addition, some tags may also affect receptor distribution within the cell itself ((Wang et al., 2008) and see detection section below). Furthermore, each cell line will express different types and levels of G proteins and regulatory proteins that will influence the outcome of the study. For example, delta opioid receptor internalization was found to be regulated by different proteins in rat primary neuronal cultures versus HEK 293 cells (Charfi et al., 2014). Also, microarray analysis has revealed several differences in gene expression of GPCRs and different signaling molecules between HEK 293 cells, AtT20, BV2 and N18 cell lines (Atwood et al., 2011). Moreover, transfection can often result in overexpression of the receptor, which also alters receptor regulation and function. Expression level may also vary across passages or batches of cells and across labs.

As an alternative to recombinant cells, there are neuronal lines that endogenously express the delta opioid receptor that may be considered closer to an endogenous environment. However, the lack of a tag means that receptor detection is more restricted. A further caveat of the use of immortalized hybrid cell lines is that they tend to lose chromosomes as they divide (Heumann et al., 1977; Geraghty et al., 2014). Therefore, it is recommended to create a large stock of frozen cells of low passage number, strictly track the number of passages, use the hybrid cells in a defined bracket of passage numbers, and report which passages were used for publication. The genetic instability of these cells lines can lead to batch variability and could contribute to variations in results between different laboratories. Some of the most commonly used cells lines that endogenously express the delta opioid receptor are the following:

*NG108-15 cell line.* These cells were obtained by inactivated Sendai virus-induced fusion of mouse N18TG2 neuroblastoma cells with rat C6-BU-1 glioma cells (Klee and Nirenberg, 1974). They served as a source for the initial cloning of the delta opioid receptor (Evans et al., 1992; Kieffer et al., 1992), and have long been used as a cellular model for this receptor (Law et al., 1982, 1983; Hsia et al., 1984; Moses and Snell, 1984; Cone et al., 1991; Roerig et al., 1992). They carry a homogenous population of endogenous delta opioid receptor, and do not express mu or kappa opioid receptors (Chang and Cuatrecasas, 1979). However, they do express the opioid receptor-like receptor (ORL1 (Ma et al., 1997)). Importantly, NG108-15 cells rely to a large extent on anaerobic glycolysis for energy production. This gives rise to high concentrations of lactic acid, producing cell dissociation and loss of viability (Hamprecht et al., 1985). A further important issue to keep in mind is the instability of delta opioid receptor expression in this line; where early passages have higher probability of expressing the delta opioid receptor (passage number $< 30$).

*F11.* This is a hybrid cell line created from the fusion of embryonic rat dorsal root ganglia (DRG) neurons with cells from mouse neuroblastoma N18TG2 (Platika et al., 1985). These cells exhibit properties characteristic of DRG neurons including neuronal morphology, expression of cell-surface gangliosides and excitable membranes (Platika et al., 1985). They express mu and delta opioid



Please cite this article in press as: Vicente-Sanchez A et al. The delta opioid receptor tool box. Neuroscience (2016), http://dx.doi.org/10.1016/j.neuroscience.2016.06.028