

## New Insights on Expression and Function of Mu and Delta Opioid Receptors in Mouse Gastrointestinal Tract



Opioids and derivatives, which target cognate receptors in the central and peripheral nervous systems, are the most widely used drugs for managing pain. Unfortunately, opioids are generally ineffective in managing visceral abdominal pain largely because of the severe side effects from targeting the enteric nervous system (ENS), including nausea, vomiting, constipation, and delay in gastrointestinal (GI) transit.<sup>1</sup> This is a significant drawback because visceral pain is the leading complaint from patients with irritable bowel syndrome (IBS), which affects up to 20% of the U.S. population. Indeed, management of IBS-related visceral pain remains an important unmet clinical need.

Activation of mu opioid receptors (MORs) and delta opioid receptors (DORs) by agonists has similar modulatory functions on enteric neurons, ie, suppressing neuronal excitability and transmitter release by hyperpolarizing the membrane potential close to the potassium equilibrium potential. The discovery that MORs and DORs can form heteromers<sup>2</sup> triggered the design of new pharmacologic molecules that affect MORs and DORs simultaneously. Following that concept, eluxadoline (Viberzi) was developed to have mixed effects on these receptors, functioning as a MOR agonist and a DOR antagonist. DOR antagonism was developed to augment analgesic effects of MOR activation while limiting constipation caused by MOR-mediated inhibition of motility. Eluxadoline was approved in the United States in 2015 to alleviate symptoms in patients with diarrhea-predominant IBS, including visceral pain.<sup>3</sup> Despite the clinical success of eluxadoline, several fundamental questions regarding the interaction between the MORs and DORs in the ENS remain unresolved. First, the level of coexpression of MORs and DORs in the submucosal and myenteric plexuses remains inconclusive because antibody-based detection of opioid receptors is known to be affected by specificity and low level of detection. Second, we still lack the understanding of the functional interaction between the MORs and DORs in the GI tract at the cellular and physiological level. Third, it is unclear whether heteromerization of MORs and DORs contributes to their functional interactions in the GI tract.

The study by DiCello et al<sup>4</sup> published in the current issue of *Cellular and Molecular Gastroenterology and Hepatology* addresses the above questions by using genetic knock-in mice, in vitro pharmacologic tests on colon strips, and in vitro and in vivo internalization assays of opioid receptors. To avoid concerns on specificity of antibodies against opioid receptors, the authors used a genetic knock-in mouse strain in which MORs and DORs are genetically tagged by fluorescent proteins mCherry and eGFP, respectively. The same mouse strain was used in prior studies by others to determine the expression patterns of MORs and DORs in peripheral sensory afferents<sup>5</sup> and in the central

nervous system.<sup>6</sup> These studies reported very limited coexpression of MORs and DORs in peripheral afferents, spinal neurons, and brain regions. In contrast, DiCello et al reported significant coexpression of MORs and DORs in the ENS of the GI tract, ie, in 22%–30% of the myenteric neurons immunopositive for Hu. MOR is primarily expressed in neurons in the myenteric plexus but is not expressed by key non-neuronal cell types relevant to intestinal motility. MORs and DORs are expressed at comparable levels in excitatory and inhibitory myenteric neurons, suggesting that activation of MORs and DORs can have both inhibitory and excitatory effects (via disinhibition) on colonic smooth muscle tone.

Following these studies, the authors performed pharmacologic assays with subtype-specific agonists/antagonists of MORs and DORs to assess the effects of each on colonic smooth muscle tones. Both MOR and DOR agonists evoked tonic muscle contraction via disinhibition, which could be desensitized by pretreatment of DOR agonists but not by pretreatment of MOR agonists. These outcomes suggest the functional interactions between MORs and DORs in mediating colonic smooth muscle tone. The MOR and DOR agonists both inhibited electrically evoked (neurogenic) muscle contraction of the colon strips, likely through actions on cholinergic excitatory ENS neurons. Interestingly, the MOR agonist-induced inhibition of muscle contraction was not affected by the DOR antagonist at a selective concentration, and the DOR agonist-induced inhibition was also not affected by the MOR antagonist. It was previously reported that the formation of MOR-DOR heteromers led to allosteric interactions between individual receptors, resulting in modulatory effect on one receptor when antagonizing the other.<sup>7</sup> The observation by DiCello et al of an absence of effects by either DOR or MOR antagonists suggests the lack of heteromer formation between MORs and DORs in mouse colon. The authors further confirmed the lack of MOR-DOR heteromers by conducting receptor internalization assays, which showed the absence of cointernalization of DORs and MORs. Collectively, these studies provide solid experimental evidence to support a functional interaction between MORs and DORs in mouse myenteric neurons without forming MOR-DOR heteromers.

The article by DiCello et al has significantly advanced our understanding of the interaction between MORs and DORs in mouse intestine. It has also raised several further questions that warrant future research and studies. Regarding the functional interactions between the DORs and MORs, it remains undetermined on the exact biochemical pathways that underlie this one-way desensitization of MOR by DOR agonists, but not the other way around. Also, MOR is not abundantly expressed in the submucosal plexus according to the mCherry fluorescent pattern, which has raised

questions on the level of participation by MOR in modulating epithelial function such as intestinal secretion. In addition, tagging the MOR with mCherry protein appears to have affected the protein trafficking because the mCherry signals are absent in neurites or nerve fibers in contrast to the immunostaining patterns by MOR antibodies. Thus, it remains undetermined whether the MOR and DOR expression patterns in those protein-tagged knock-in mice and hence their activities are comparable with wild-type mice.

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### Conflicts of interest

The author discloses no conflicts.



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