Constitutive Internalization and Recycling of the Delta opioid Receptor

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Abstract

The complex and dynamic interplay between internalization, anterograde transport, recycling and degradation determines the density of functional G protein-coupled receptors (GPCRs) at the cell surface and, consequently, the magnitude of their associated physiological responses. As opposed to most members of the GPCR superfamily, the delta opioid receptor (DOP) is only weakly expressed at the neuronal plasma membrane, thus representing a critical limitation for its use as a therapeutic target. Although DOP appears as a promising candidate for the development of better-tolerated analgesics, the molecular and cellular mechanisms underlying the regulation of its cell surface expression remain poorly characterized. This work investigates the constitutive (i.e. ligand-independent) trafficking of DOP, an understudied cellular process potentially involved in the control of plasma membrane-localized receptors. In HEK293 cells stably expressing Flag-tagged DOP, we first confirmed that this GPCR is constitutively internalized through a clathrin-dependent and β-arrestin-independent mechanism. Immunofluorescence experiments with selected Rab protein isoforms indicated that internalized DOP was mainly colocalized with the early endosome marker Rab5, as well as the rapid recycling endosome marker Rab4. Co-transfection with Rab5 dominant-negative mutant inhibited the intracellular distribution of the receptor, indicating that its constitutive endocytosis is Rab5-dependent. DOP cell surface expression and ligand-induced signaling were also significantly reduced following Rab4-specific DsiRNA treatments, suggesting a role for this small GTPase in the regulation of DOP constitutive recycling. Mapping of the major region of interaction between DOP and both Rabs revealed that Rab4 binds the third intracellular loop of DOP, whereas Rab5 seems to preferentially interact with the distal region of the C-terminal end of DOP. Altogether, these results show for the first time that DOP constitutive internalization and recycling are critical to maintain its cell surface bioavailability and responsiveness to agonists.

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GRAPHICAL ABSTRACT

Constitutive trafficking of the DOP

Delta opioid receptor (DOP)

Clathrin

AP-2 complex

Direct DOP-Clathrin adaptor?

Rab5

Early Endosome

Recycling Endosome

Rab4
FIGURE 1

A

B

% of Flag-DOP internalization

Time (min)

0  30  60  120

**  ***  ****
FIGURE 7

A

GST  GST-DOP-ICL1  GST-DOP-ICL2  GST-DOP-ICL3  GST-DOP-CT

25 kDa

Binding Reaction IB:HA (Rab4)

37 kDa

25 kDa

Input IB:HA (Rab4)

B

GST  GST-DOP-ICL1  GST-DOP-ICL2  GST-DOP-ICL3  GST-DOP-CT

37 kDa

Binding Reaction IB:HA (Rab5)

25 kDa

37 kDa

Binding Reaction IB:GST

25 kDa

Input IB:HA (Rab5)

C

Extracellular

Intracellular

ECL3

TM7

G-term

330-STOP

354-STOP

37 kDa

Binding Reaction IB:HA (Rab5)

25 kDa

37 kDa

Binding Reaction IB:GST

25 kDa

Input IB:HA (Rab5)
### TABLE S1 – siRNA and DsiRNA sequences

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<th>DsiRNAs</th>
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