NOP Receptor Signaling Cascades

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Contents

Abstract

The nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor is a G protein-coupled receptor with wide distribution throughout the peripheral and central nervous system. Similar to other opioid receptors, NOP receptors couple to intracellular second messengers and regulatory proteins to affect biological systems. In this chapter, we review the current literature for NOP signaling cascades including their role as classic GPCRs, the investigation of their kinase and arrestin signaling pathways, and the importance of examining biased signaling to critically evaluate the therapeutic potential of novel NOP agonists.

Keywords

Arrestin · Bias · G protein · N/OFQ · Nociceptin · NOP receptors

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1 Classic Gi-Signaling Pathways

Similar to all GPCRs, nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor couples to the inhibitory G protein $G\alpha_{i/o}$, and following agonist stimulation, the G protein exchanges GDP for GTP and permits Gα and Gβγ subunits to dissociate and act on the various intracellular pathways (Childers and Snyder 1978; Childers et al. 1979). Early research in opioid receptor pharmacology revealed that guanine nucleotides, such as GTP, control agonist binding to opioid receptors in membrane preparations from brain tissue (Blume et al. 1979). Later, Barchfeld and Medzihradsky (1984) determined that opioid agonists stimulate GTPase activity, while other groups determined that NOP receptor activation distinctly promotes guanine nucleotide exchange (Sim et al. 1996; Narita et al. 1999). Similar to the manner of other GPCRs, agonist stimulation of opioid receptors was also shown to reduce cyclic adenosine monophosphate (cAMP) production. Several studies confirmed that NOP receptor activation inhibits adenylyl cyclase activity, and it is broadly accepted that the NOP receptor couples to pertussis toxin-sensitive G proteins, including Gαi, to initiate inhibition of cAMP formation (Meunier et al. 1995; Butour et al. 1997; Zhang et al. 2012). Indeed, the initial identification of endogenous NOP ligand, N/OFQ, did so based on N/OFQ's ability to inhibit cAMP. Collectively, this inhibition reduces cell regulatory signaling through decreased activity of cAMP-dependent protein kinase, as well as cell proliferation and gene regulation.

It has also been suggested that NOP receptors can couple to other G proteins, G_Z and G_{16}. This noncanonical NOP G protein signaling has been less well characterized in physiologically relevant systems and has only been demonstrated in heterologous expression studies and SH-SY5Y cells (Chan et al. 1998). Similar to canonical opioid receptors, NOP receptors couple to Kir3 and Ca2+ channels via Gβγ pathways (Connor et al. 1996; Connor and Christie 1998). Channel deactivation for Kir3 interactions happens after GTP to GDP hydrolysis and Gβγ removal from interaction with the channel (Wickman and Clapham 1995). This opening of Kir channels causes cellular hyperpolarization and inhibits tonic neural activity. NOP receptors have also been shown to reduce Ca2+ currents sensitive to P/Q-type, N-type, and L-type channel blockers when activated (Connor et al. 1996; Zhang et al. 2012). Specifically, NOP receptor inhibition of N-type calcium conductance is likely mediated by binding of the dissociated Gβγ subunit directly to the channel and reduces voltage activation of channel pore opening (Zamponi and Snutch 1998, 2002; Beedle et al. 2004; Yeon et al. 2004; Ruiz-Velasco et al. 2005). Recently, it has also been shown that NOP receptors use Rho-associated coiled-coil-containing protein kinase (ROCK) and LIM domain kinase (LIMK) in the regulation of voltage-dependent Ca2+ channels (Mittal et al. 2013).

2 NOP Receptors and Kinase Signaling

As all GPCRs couple to various intracellular kinase cascades, opioid receptors have been shown to couple to protein kinase A (PKA) and protein kinase C (PKC) pathways as well as signaling through mitogen-activated protein kinase (MAPK) cassettes. In general, these pathways are key regulatory mechanisms within cellular signaling that control diverse physiological outcomes. Beginning in the 1990s, investigators learned that the phosphorylated arrestin-bound GPCR complex is not simply inactive but that it recruits alternate signal transduction cascades, including MAPKs (Fukuda et al. 1997; Hawes et al. 1998; Bruchas and Chavkin 2010; Whalen et al. 2011; Chang and Bruchas 2014). Similarly, signaling to MAPK cassettes in opioid receptors and NOP receptors can be mediated through this process (Zhang et al. 2012). NOP receptor activity can induce activation of PKC (Armstead 2002) as well as activation of phospholipase A2 and C (Fukuda et al. 1998; Yung et al. 1999). NOP receptor-dependent activation of all three MAPK cascades (ERK1/ERK2, p38, and JNK1/JNK2/JNK3) has also been demonstrated. These pathways are of significant importance as the ERK1/ERK2 pathway communicates signaling that facilitates cell proliferation, cell cycle progression, cell division, and differentiation. Further, these cascades regulate apoptosis, transcription factor regulation, ion channel regulation, neurotransporter regulation, and protein scaffolding (Raman et al. 2007). Both JNK and p38 signaling pathways are responsive to cellular stressors, such as cytokines, ultraviolet irradiation, heat shock, and osmotic shock, and are involved in adaptation to stress, apoptosis, or cell differentiation (Raman et al. 2007). Although NOP receptor-induced extracellular signal-regulated kinase (ERK) phosphorylation has not been extensively studied, endogenous agonist N/OFQ has been demonstrated to cause NOP receptor-mediated increases in ERK1/ERK2 phosphorylation levels in heterologous expression systems (COS7, CHO, and HEK293 cells) (Lou et al. 1998; Zhang et al. 2012). Recently, it was reported that ERK1/ERK2 signaling via NOP receptors was independent of receptor phosphorylation and GRK/arrestin signaling (Zhang et al. 2012). However, further examination within alternate model systems and with other ligands is warranted.

Recently, opioid receptor activation of p38 MAPK cassettes has gained attention due to the effects of kappa receptor-induced p38 phosphorylation and resulting aversion-like behaviors (Bruchas and Chavkin 2010; Bruchas et al. 2011). Similar to kappa receptors, NOP receptor activation has been linked to this phosphorylation of p38 MAPK in vitro as Zhang et al. (1999) demonstrated that NOP receptors activate p38 signaling via protein kinase A and PKC pathways in NG108-15 cells. However, further examination of NOP receptor-mediated p38 signaling in endogenous systems during pathologic conditions (as demonstrated in Armstead 2006) and in specific tissues will provide important insights into the coupling of NOP receptors to this MAPK cassette.

Additionally, the activation of c-Jun N-terminal kinase (JNK) signaling by opioid receptors has been recently examined for its interesting mu and kappa regulatory properties (Bruchas et al. 2007; Melief et al. 2010; Al-Hasani and Bruchas 2011). For the NOP receptor, important early studies in NG108 cells showed that N/OFQ

could induce phosphorylation of JNK in a time- and concentration-dependent manner (Chan and Wong 2000). This report suggested that JNK activation via NOP receptors could occur in a pertussis toxin (PTX)-sensitive and pertussis toxin (PTX)-insensitive manner. PTX-insensitive G proteins, G_Z, G12, G14, and G16, were all reported to potentially play a role (Chan and Wong 2000). Moreover, PTX-insensitive NOP-mediated JNK signaling was determined to be mediated through G protein-coupled receptor kinase 3 (GRK3) and arrestin-3 as late-phase JNK phosphorylation was absent following selective siRNA knockdown of GRK and arrestin (Zhang et al. 2012). Additionally, this report confirmed this GRK-/arrestin-mediated effect using cells expressing a C-terminal phosphorylation NOP receptor mutant (S363A) and also corroborated reports that NOP receptors couple to JNK in a PTX-sensitive fashion during the early phase of activity.

3 NOP Receptors and Arrestin Signaling

GPCR internalization is mediated through recruitment of arrestin and typically via either a clathrin-dependent or clathrin-independent process. Similar to other opioid receptor subtypes, phosphorylation by GRK2 or GRK3 of the NOP receptor leads to arrestin-2 or arrestin-3 recruitment (Zhang et al. 2012). This arrestin-2 and arrestin-3 binding modulates NOP receptor desensitization and ultimately assists in determining receptor status.

Several groups have examined the many stages of NOP receptor trafficking (for review, see Donica et al. 2013). Although initial study in the NOP receptor field had difficulty demonstrating agonist-induced internalization (Dautzenberg et al. 2001), Spampinato et al. (2001, 2002, 2007) clearly demonstrated that N/OFQ treatment induces NOP internalization. Similar to the kappa opioid receptor disparities in internalization conditions (Bruchas and Chavkin 2010), differences reported in the internalization of NOP receptors are likely due to expression variability and the model system implemented. Indeed, most studies implicating arrestin in this signaling have been conducted in heterologous expression systems using overexpressed arrestins and opioid receptor subtypes. Indeed, knockdown of arrestin-3, but not arrestin-2, blocks NOP receptor internalization after treatment with N/OFQ (Zhang et al. 2012). When Ser363, a putative GRK phosphorylation site on the NOP receptor, was mutated to an alanine, arrestin-3 was not recruited to the cell surface after N/OFQ treatment and the mutant S363A demonstrated significantly reduced NOP receptor internalization (Zhang et al. 2012). Similarly, the dominant positive arrestin-3-(R170E), which does not require receptor phosphorylation, was able to rescue a NOP receptor S363A mutant's internalization (Zhang et al. 2012). A recent study has also demonstrated that NOP receptors use arrestin-2 to regulate downstream signaling (Mittal et al. 2013). Here investigators demonstrated enhanced NOP function in dorsal root ganglia neurons that lacked arrestin-2. Using patch clamp, whole-cell recording, the authors found that nociceptin has greater inhibition of voltage-dependent Ca^{+2} channels in arrestin-2 KO mice compared to wild-type mice. Further, they demonstrated that NOP agonist Ro 65-6570 administration

produces a hypolocomotor response in arrestin-2 KO mice while having no effect in wild-type mice. Currently, investigators are examining how the NOP receptor engages these various arrestins and whether agonists with varying efficacies and potencies can induce different rates of internalization and divergent arrestin-2 and arrestin-3 recruitment (Chang et al. 2015; Malfacini et al. 2015). Recently, evidence suggests that compounds acting as partial agonists with respect to NOP/G protein signaling behave as antagonists with little to no activity in NOP/arrestin coupling (Chang et al. 2015; Malfacini et al. 2015; Asth et al. 2016). In fact, although NOP receptors functionally recruit both arrestin-2 and arrestin-3, arrestin-3 recruitment is likely more efficacious (Chang et al. 2015). Many NOP ligands also differ in the kinetics of arrestin recruitment as demonstrated using bioluminescence resonance energy transfer (BRET) techniques (Chang et al. 2015). Certainly, it is possible that agonist, cell type, and environment have a significant impact on NOP internalization and arrestin recruitment properties (Malfacini et al. 2015). Indeed, Asth et al. (2016) demonstrated that some NOP ligands can have similar G protein interaction yet have divergent arrestin recruitment (partial agonism vs antagonism) that drive behaviorally relevant outcomes for anxiety and depression. Based on these initial reports, it may be possible to further design arrestin-biased NOPR ligands in figure efforts (Fig. 1).

In most cases, NOP receptor internalization starts rapidly, within 5–10 min after agonist treatment, with very robust internalization at 1 h posttreatment in transfected cells (Spampinato et al. 2001; Corbani et al. 2004; Zhang et al. 2012). As with other

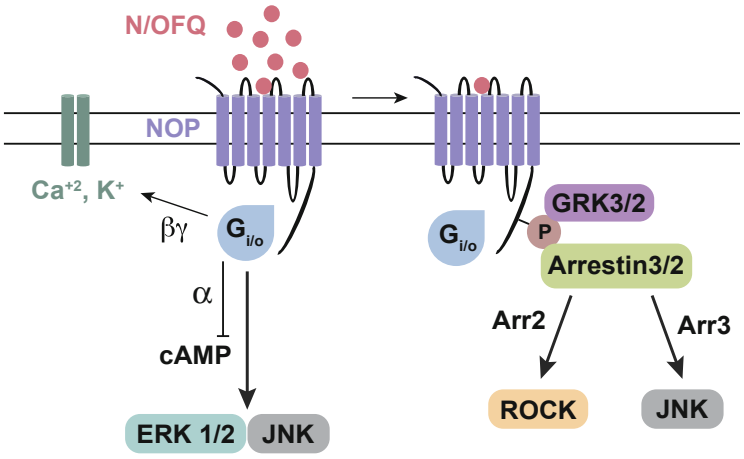

Fig. 1 Summary of NOP receptor signaling: Figure highlights basic NOP receptor signal transduction and trafficking pathways and features canonical NOP receptor coupling to inhibition of calcium channels and activation of inward rectifying potassium channels (Connor et al. 1996; Connor and Christie 1998). Figure additionally highlights reports of NOP receptor activation of MAPKs and desensitization mechanisms via GRK3 and GRK2 (Zhang et al. 2012). Figure also depicts findings that NOP receptor activation can initiate downstream signaling to JNK and ROCK pathways via arrestin signaling (Zhang et al. 2012; Mittal et al. 2013). Arrows refer to activation; T lines refer to block or inhibition of function. Figure adapted from Toll et al. (2016)

opioid receptor subtypes, the level of internalized receptor depends on the ligand. However, hexapeptide partial agonists do not induce receptor internalization or robust GRK translocation (Spampinato et al. 2001; Corbani et al. 2004). This could be due to their partial agonism as other NOP partial agonists such as [F/G] N/OFQ(1–13)-NH2 also lack receptor internalization or due to hexapeptides having a different NOP-binding site (Bes and Meunier 2003). It has been proposed that receptor regulation is dependent on the specific agonist examined and that peptides and small molecule agonists may impact the regulation of NOP receptors via different mechanisms (Donica et al. 2013; Chang et al. 2015; Malfacini et al. 2015; Asth et al. 2016).

N/OFQ via selective NOP receptor activation can control several biological functions; however the relative role of G protein and arrestin in mediating these actions is not completely understood. It is known that other ligands may act as biased agonists at the NOP receptor since they can have different efficacies in activating G protein versus arrestin pathways. Recent investigation has used BRET assays to assess multiple ligands to determine these distinct biases and have demonstrated the diverse impact NOP ligands have in these signaling pathways (Chang et al. 2015; Malfacini et al. 2015; Ferrari et al. 2016, 2017; Rizzi et al. 2016). This distinction is critical to dissecting the biological actions that follow the activation of this receptor as these biased ligands may act as more effective therapeutics. Critically, studies suggest that actions on arrestin signaling, rather than G protein efficacy, may be a better predictor of behavioral outcomes in vivo (Asth et al. 2016). Comparing in vitro and in vivo actions, it appears that NOP ligands able to promote NOP/arrestin-3 interaction (N/OFQ, Ro 65-6570, and AT-090) are also able to induce anxiolytic-like effects in an elevated plus maze test (Asth et al. 2016). However, compounds that inhibit NOP/arrestin-3 interaction (UFP-101, SB-612111, UFP-113, and [F/G]N/OFQ(1–13)-NH2) produced antidepressant-like effects in a forced swim test. Given this critical divergence, thorough in vitro and in vivo investigation of full and partial agonists and pure antagonists is required to elucidate their therapeutic potential.

New studies that reveal the signaling profiles of NOP receptor ligands previously only classified as agonists, antagonists, inverse agonists, or partial agonists offer the prospect to connect these biases to observed biological effects and better understand NOP receptor function. Further studies are also needed to identify new lead molecules that will help to understand the structural requirements underlying the difference in efficacy of NOP agonists for G proteins and arrestins and the potential therapeutic indications of G protein- or arrestin-biased NOP agonists.

4 Conclusion

This chapter intends to describe the extensive effort made to illuminate the NOP receptor's cellular signaling pathways. As the most recently unveiled opioid receptor, this avenue of research continues to be of significant importance for researchers who aim to utilize this receptor in the development of novel drug therapeutics for the

treatment of pain, substance use, and psychiatric disorders. The divergent activation of G protein-biased and arrestin-biased pathways is particularly informative as understanding distinct differences in agonists' propensity to activate these pathways will guide investigation to feasible therapeutic interventions with minimal side effects.

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