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The Orphanin FQ/Nociceptin (OFQ/N) System

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Abstract Orphanin FQ/nociceptin (OFQ/N) was the first novel neuropeptide discovered as the natural ligand of an orphan G protein-coupled receptor (GPCR). Orphan GPCRs are proteins classified as receptors on the basis of their sequence similarities to known GPCRs but that lack the ligands that activate them in vivo. One such orphan GPCR exhibited sequence similarities with the opioid receptors. OFQ/N was isolated as its natural ligand and shown to also share sequence similarities to the opioid peptides. This led to numerous studies attempting to find functional similarities and differences between the OFQ/N and opioid systems. This chapter will summarize our knowledge of the OFQ/N system and of its roles in the organism.

Keywords Anxiety · Drug dependence · Memory · Neuropeptide · Orphan GPCR · Pain · Stress

Abbreviations

OFQ/N	Orphanin FQ/nociceptin
GPCR	G protein-coupled receptor
NOP	OFQ/N receptor

1 Discovery of OFQ/N

The G protein-coupled receptor (GPCR) ORL-1, also called XOR-1, ROR-C, LC132, Hyp8-1, and C (Bunzow et al. 1994; Chen et al. 1994; Fukuda et al. 1994; Lachowicz et al. 1995; Mollereau et al. 1994; Wang et al. 1994; Wick et al. 1995), had been found through its similarities to the opioid receptors and is known to bind none of the opioid peptides or synthetic opiates (Bunzow et al. 1994; Mollereau et al. 1994; Wang et al. 1994). Its natural ligand was therefore unknown, making it part of the orphan GPCRs.

Purification of the natural ligand of the ORL-1 receptor was achieved simultaneously in two different laboratories starting from either rat or porcine brain extracts (Meunier et al. 1995; Reinscheid et al. 1995). ORL-1 cDNA was transfected into cells that were exposed to the different extracts. Because of its similarities to the opioid receptors, it was assumed that ORL-1 might also bind a peptidergic ligand and share the same coupling mechanism to second

messenger systems as that of the opioid receptors, i.e. inhibition of adenylyl cyclase activity. Extracts were fractionated and active fractions were further purified. Both approaches led to the identification of a 17-residue long peptide with the primary structure FGGFTGARKSARKLANQ. This peptide was named orphanin FQ (OFQ, to mark its origin or nociceptin) (Reinscheid et al. 1995) (N, because of its hyperalgesic activity) (Meunier et al. 1995). Consequently, the ORL1 receptor was classified as the fourth member of the opioid receptor family and renamed OFQ/N peptide (NOP) receptor by NC-IUPHAR.

2 Pharmacology and Cellular Responses Induced by OFQ/N

OFQ/N was first shown to inhibit forskolin-stimulated cAMP accumulation in NOP-transfected cells with a median effective concentration of 1.05 ± 0.21 nM and a maximal effect of ~80% inhibition at 100 nM (Meunier et al. 1995; Reinscheid et al. 1995). To investigate its binding constants, a radioligand was developed (Reinscheid et al. 1995). Because OFQ/N does not contain Tyr residues, a series of Tyr-substituted peptide analogs were synthesized. The Tyr^{14}-substituted OFQ/N was shown to be an agonist with equivalent potency in cAMP assays as the unsubstituted OFQ/N (EC_{50} values of 1.02 ± 0.11 nM). The ^{125}I-labeled Tyr^{14}-substituted peptide displayed saturable, displaceable and reversible binding to membranes of opioid-like orphan receptor transfected cells with a K_d of 0.1 ± 0.02 nM (Reinscheid et al. 1995). Its binding constants are well in the range of affinities observed for other neuropeptides.

Within a short time of its discovery, the OFQ/N receptor was shown to induce a variety of intracellular effects. First, as described above, OFQ/N receptor was shown to induce an inhibition of adenylyl cyclase in CHO cells transfected with the NOP receptor (Meunier et al. 1995; Reinscheid et al. 1995). Next, modulation of cellular excitability was detected when OFQ/N was found to increase inwardly rectifying K-conductance in dorsal raphe nucleus neurons (Vaughan et al. 1996) and in the arcuate nucleus (Wagner et al. 1998); to increase K-conductance in periaqueductal gray neurons (Vaughan et al. 1996) and in locus coeruleus neurons (Connor et al. 1996a); to couple to G protein-activated K channels (Ikeda et al. 1997); to inhibit voltage-gated calcium channels in freshly dissociated CA3 hippocampal neurons (Knoflach et al. 1996); to inhibit T-type Ca channels in sensory neurons (Abdulla et al. 1997); and to inhibit N-type Ca channels in SH-SY5Y cells (Connor et al. 1996b). Also, the OFQ/N receptor appears to couple to K channels in Xenopus oocytes (Matthes et al. 1996). Furthermore, OFQ/N has been shown to inhibit the release of glutamate and GABA from nerve terminals (Faber et al. 1996; Nicol et al. 1996), to block acetylcholine release from retina (Neal et al. 1997) and parasympathetic nerve terminals (Patel et al. 1997), to inhibit synaptic transmission and long-term potentiation in the hippocam-

pus (Yu et al. 1997), to suppress dopamine release in the nucleus accumbens (Murphy et al. 1996), and to inhibit tachykinin and calcitonin gene-related peptide release from sensory nerves (Giuliani et al. 1996; Helyes et al. 1997). OFQ/N was demonstrated to activate mitogen-activated protein kinase in receptor transfected CHO cells (Fukuda et al. 1997). Together, these results show that OFQ/N is able to modulate the biochemical properties of cells, alter the electrophysiological properties of neurons and to affect their transmitter release. In organotypic assays, OFQ/N has been shown to inhibit electrically induced contractions of the vas deferens, ileum, and myenteric plexus preparations (Berzetei-Gurske et al. 1996; Calo et al. 1996; Nicholson et al. 1998; Zhang et al. 1997). Importantly, none of the effects of OFQ/N described were inhibited by opiate antagonists, emphasizing the pharmacological difference between the opioid and the OFQ/N systems (see below).

These data exemplify the breadth of responses that the OFQ/N system may modulate, and merely underscore the fact that the expression of the NOP receptor in a particular neuronal system is sufficient to expect that OFQ/N may modulate this system.

3 Synthesis and Inactivation of OFQ/N

Like all bioactive peptides, OFQ/N is synthesized as part of a larger polypeptide precursor (preproOFQ/N, ppOFQ/N), which has been cloned from rat, human (Mollereau et al. 1996; Nothacker et al. 1996), mice (Saito et al. 1996), and bovine (Okuda-Ashitaka et al. 1998). The primary structure of the precursor protein contains the typical structural elements of a neuropeptide precursor. It starts with an amino terminal signal peptide necessary for its secretion. The OFQ/N sequence is flanked by pairs of Lys-Arg residues, indicating that its maturation requires trypsin-like cleavages (Fig. 1).

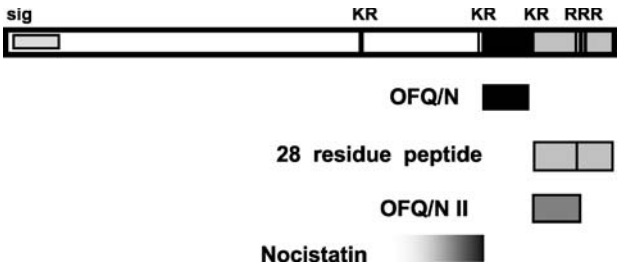

Fig. 1 Synthesis of OFQ/N, showing the structure of the OFQ/N precursor. The bioactive peptides potentially generated by processing are described relative to their corresponding location in the precursor. The nocistatin N-terminus is not described due to its lack of identity in different species. *Sig* signal peptide, *K* Lys, *R* Arg

But, these are not the only putative sites for precursor processing. The C-terminus of the precursor protein downstream of the OFQ/N sequence is conserved among the different species genes and could generate either a 28-residue long peptide or, after cleavage at the Arg triplet, a 17-residue long peptide, whose terminal amino acids are the same as these of OFQ/N (Fig. 1). These two peptides were synthesized but were unable to either bind or activate the NOP receptor. It has been reported that the 17-amino acid peptide (termed NocII or OFQ II) exhibits some effect on locomotion and pain perception (Florin et al. 1997b; Rossi et al. 1998).

The bovine precursor harbors an additional pair of basic amino acids N-terminal to the OFQ/N sequence that could give rise to a 19-amino acid peptide (Okuda-Ashitaka et al. 1998). This putative was isolated from bovine bran and has been reported to possess an anti-OFQ/N activity because it was able to block OFQ/N-induced allodynia and hyperalgesia (Okuda-Ashitaka et al. 1998). This peptide was named nocistatin (Fig. 1) and acts via a receptor different from the NOP receptor. The active part of nocistatin was found to reside in its C-terminal hexapeptide that is also the only conserved structure between all mammalian OFQ/N precursors. The bovine form of nocistatin appears to be species-specific because the human, rat, mouse, and porcine precursor lack the pair of basic amino acids that is used for processing in the bovine precursor protein. The fact that OFQ II or nocistatin require their own receptors but that none have been identified that would exhibit selectivity for these two peptides indicate that these peptides may not be neuropeptides that act through a traditional GPCR system.

Inactivation of OFQ/N has also been studied. Metallopeptidases play a major role. The critical sites of enzymatic cleavage are Phe1–Gly2, Ala7–Arg8, Ala11–Arg12, and Arg12–Lys13 bonds. Aminopeptidase N and endopeptidase 24.15 are the two main enzymes involved in OFQ/N metabolism. Endopeptidase 24.11, which is involved in enkephalin catabolism, does not appear to be critically involved (Montiel et al. 1997; Noble et al. 1997).

4 The OFQ/N and Opioid Systems

There are striking sequence similarities between the OFQ/N and opioid systems. First, the N-terminus of OFQ/N, FGGF, is highly reminiscent of the canonical YGGF of the opioid peptides. Then, the NOP receptor shares more than 60% identity with the three opioid receptors (Bunzow et al. 1994; Mollereau et al. 1994). Furthermore, the OFQ/N precursor protein exhibits several analogous structures as compared to the opioid precursors: the active peptides are located in the C-terminal part and seven Cys residues are found conserved at the N-terminus of ppOFQ/N, prodynorphin, and proenkephalin (Mollereau et al. 1996; Nothacker et al. 1996). Finally, the OFQ/N precursor

gene has retained an intron–exon organization similar to that of the opioid precursor genes (Mollereau et al. 1996)]. The coding sequence is divided over two exons, the smaller one containing the translational start site (AUG), the other encoding the rest of the open reading frame. From this common architecture of the opioid precursor genes, the OFQ/N precursor gene differs in that it contains an additional exon for the 3′-untranslated region of the mRNA. In humans, the OFQ/N gene has been mapped to the chromosomal location 8p21 (Mollereau et al. 1996). Altogether, these data support the view that the receptors, as well as the neuropeptide precursors of both the opioid and the OFQ/N systems, have evolved from common ancestral genes.

While the OFQ/N and opioid systems share significant similarities at the sequence level it has been shown that OFQ/N does not activate opioid receptors nor do the opioid peptides elicit biological activity at the OFQR. The basis for this selectivity is inherent to the primary structures of OFQ/N and the opioid peptides. A series of truncated and/or chimeric peptides led to the conclusion that OFQ/N and dynorphin A, its closest counterpart, contain domains that specifically act to prevent cross-activation of other but their own receptors (Reinscheid et al. 1998). These domains are composed of single residues in key positions together with short stretches of amino acids that do not overlap in both peptides (Fig. 2). It has been further demonstrated that by mutating as few as four amino acids, a receptor can be produced that recognizes dynorphin with very high affinity and yet still binds OFQ/N as well as the wild-type receptor. This indicates that the NOP has evolved features that specifically exclude opioid binding and that these features are distinct from those required for the binding of OFQ/N (Meng et al. 1996) Together these

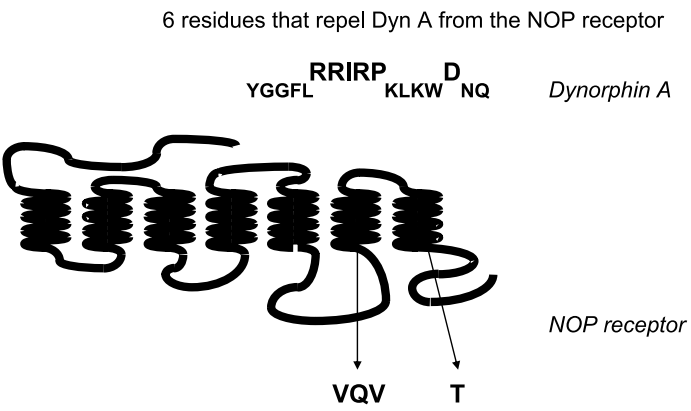

Fig. 2 Configuration of the NOP receptor and of dynorphin A. Dynorphin A's residues *RRIRP* and *D* have been shown to repel dynorphin A from the NOP receptor, while NOP's residues *VQV* and *T* prevent dynorphin A from binding (see text)

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