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Review

Nociceptin/orphanin FQ and the opioid receptor-like ORL1 receptor

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Abstract

Homology cloning and, more recently, the sequencing of whole genomes, have identified many open reading frames encoding proteins of unknown function, in particular putative G protein-coupled membrane receptors. Identification of orphan receptors in this way has marked the advent of 'reverse pharmacology' to identify the corresponding physiological ligands. This approach has led to the discovery of the ORL1 (Opioid Receptor-Like 1) receptor, and of its natural ligand, nociceptin/orphanin FQ (noc/oFQ), the basic components of a new peptide-based signalling pathway in the nervous sytem. Based on genetic criteria, the ORL1 and opioid receptors belong to the same family, as do noc/oFQ and opioid peptides. The marked structural analogy between the ORL1 and opioid receptors, especially the κ-opioid receptor, and the noc/oFQ and opioid peptides, particularly dynorphin A, is not reflected anatomically since noc/oFQ and opioid peptides appear to be located in separate neuronal circuits. Noc/oFQ triggers the same G protein-mediated signalling pathways as do opioids, however, to produce pharmacological effects that sometimes differ from, and even oppose, those of opioids. Noc/oFQ stimulates an outward K^+ current and/or inhibits voltage-gated Ca^{2+} channels, thereby reducing synaptic efficacy, i.e. neuronal activity. In the rat, noc/oFQ is endowed with supraspinal pronociceptive/anti-opioid properties (it suppresses opioid-mediated analgesia), while convergent electrophysiological and behavioural data indicate that the peptide is a spinal analgesic. Noc/oFQ has not yet been found to precipitate withdrawal in morphine-tolerant rats. Nor does it elicit motivational effects, suggesting it lacks abuse liability. Also, by acting supraspinally, noc/oFQ impairs motor performance, suppresses spatial learning, induces feeding, and regulates basal and stress-induced release of pituitary hormones. Noc/oFQ is also active when administered intravenously, exhibiting potent smooth muscle relaxant, diuretic, and antinatriuretic properties. Last but not least, noc/oFQ appears to regulate stimulated immune function, and to be involved in neuronal differentiation. The discovery of noc/oFQ, a neuropeptide with multiple functions, will certainly improve our knowledge of brain physiology, and may find therapeutic applications, for example in the management of pain or hyponatremic and water-retaining diseases. However, given the wide distribution of noc/oFQ and its receptor, the pharmacological profile of noc/oFQ is likely to be incomplete, and other as yet unknown functions of the peptide remain to be discovered. Most helpful in this respect will be the identification of new ligands of the ORL1 receptor, particularly antagonists. If research on noc/oFQ carries on unabated at the present pace, potentially clinically interesting new compounds could become available in the not too distant future. © 1997 Elsevier Science B.V.

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1. Introduction

Opioids play a major role in the central and peripheral nervous systems, where they act to produce numerous pharmacological effects: acute effects (analgesia, respiratory depression, miosis, constipation, ..., sensation of well-being), which are likely to be neuromodulatory in origin, and reflect, in most if not all cases, the presynaptic depression of fast transmitter release, and chronic effects (tolerance and dependence), which may result from neuronal adaptive (plastic) changes such as those involved in cell learning and memory (Meunier, 1992). In practice, the unwanted side effects of opioids limit their widespread use as therapeutic agents and has turned them into a major class of abused drugs. Opioids act by stimulating specific membrane receptors, of which there are three major types, μ, δ and κ. These couple to *B. pertussis* toxin-sensitive G proteins to inhibit adenylyl cyclase and/or voltage-gated Ca^{2+} channels, or to stimulate an inwardly rectifying K^+ conductance (for reviews, see: Simonds, 1988; Dhawan et

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al., 1997). The cDNAs of the μ-, δ- and κ-opioid receptors have been recently cloned, sequenced, and found to encode highly homologous proteins with a primary structure typical for G protein-coupled membrane receptors (for reviews, see: Reisine and Bell, 1993; Kieffer, 1995; Satoh and Minami, 1995). Further attempts to clone novel opioid receptor types and/or subtypes, by hybridization screening at low stringency with opioid receptor cDNA probes, or using probes generated by selective amplification of genomic DNA with degenerate primers (Libert et al., 1989), led several laboratories to isolate a cDNA [ORL1 (Mollereau et al., 1994), ROR-C (Fukuda et al., 1994), XOR1 (Chen et al., 1994), XOR1 (Wang et al., 1994), LC132 (Bunzow et al., 1994), MOR-C (Nishi et al., 1994), C3 (Lachowicz et al., 1995), FLAT7-5EU (Wick et al., 1994), Hyp 8-1 (Wick et al., 1995), oOR (Halford et al., 1995)] encoding a protein with a primary structure analogous to those of opioid receptors. This opioid receptor-like receptor displayed no affinity for opioid ligands and remained an 'orphan' until late 1995, when two groups reported separately the isolation of its endogenous ligand, termed nociceptin (Meunier et al., 1995) or orphanin FQ (Reinscheid et al., 1995), a new member of the endorphin family (Julius, 1995). Identification of the neuropeptide nociceptin/orphanin FQ 'marked the sprouting of a new area in brain research' (Rowe, 1996), and has excited such interest, especially in laboratories already involved in opioid research, that a great deal of information on all aspects of the novel neuropeptide peptide has already accumulated. We shall review this here, indicating that the peptide acts at the molecular and cellular levels in very much the same way as opioids do, however to produce pharmacological effects that sometimes differ from, and even oppose, those of opioids. The orphan receptor is referred to hereafter as ORL1 (for Opioid Receptor-Like 1), and its endogenous ligand, noc/oFQ (for nociceptin/orphanin FQ).

2. The opioid receptor-like (ORL1) orphan receptor

2.1. cDNA and gene

Alignment of the cDNA-deduced amino acid sequences of the ORL1, μ-, δ- and κ-opioid receptors (Fig. 1) highlights the conserved regions, in particular the putative transmembrane helices and cytoplasmic loops. Sequence conservation among the four receptors is high in the second (17 out of 22 amino acid residues, 77.3%), third (16/22, 72.7%) and seventh (15/20, 75.0%) transmembrane helices, moderate in the first (11/24, 50%), fifth (13/23, 56.5%) and sixth (9/22, 40.9%), and low in the fourth (5/23, 21.7%). Amino acid sequence identities are also high in all intracellular loops: 6/7 (85.4%) in the first, 18/24 (75.0%) and 9/12 (75%) in the third and fourth, respectively, 13/22 (59.1%) in the second. All other regions, the first exofacial loop excepted (9/18, 50.0%), display extremely low ($\leq 8\%$) amino acid sequence conservation. Overall, the ORL1 receptor is equally distant (63–65% homology) to the three types of opioid receptor, but its acidic second exofacial loop makes it resemble more closely the κ- than the μ- or δ-opioid receptors. The murine ORL1 receptor (367 amino acids) is shorter than its human counterpart (370 amino acids), the difference accounted for by a three residue insertion in the N-terminal domain.

Blot hybridization analysis of RNA isolated from murine whole brain and/or various brain regions has consistently detected an ORL1 mRNA species 3.3–3.7 kb long, and two larger species of 7.3–13 and 9.5–23 kb (Chen et al., 1994; Fukuda et al., 1994; Wang et al., 1994; Wick et al., 1994), which may reflect differential splicing of a primary transcript, or the use of different polyadenylation sites. Their relative abundance varies according to the brain region examined. Indeed, splice variants of the ORL1 receptor have been identified, one in rat brain, with an insertion of 28 amino acid residues in the second exofacial loop (Wang et al., 1994), the other in mouse lymphocytes, with a deletion of the YVILR amino acid sequence close to the boundary between the first transmembrane helix and intracellular loop (Halford et al., 1995).

The ORL1 receptor gene has been partially characterized. The protein-coding sequence of the mouse gene is interspaced by two introns, one of ≈ 2.6 kb located within the codon for the first amino acid residue (Arg) of the receptor's putative first cytoplasmic loop (Nishi et al., 1994), and a short second one (81 bp), located within the codon for the sixth amino acid residue (Glu) of the putative second exofacial receptor loop (Mollereau et al., 1994; Nishi et al., 1994). The shorter intron is also found as a strech of 118 bp in the human gene (Mollereau et al., 1994). The *ORL1* gene has been mapped to the distal region of mouse chromosome 2 (Chen et al., 1994; Nishi et al., 1994), and to the q13.2–13.3 region of human chromosome 20 (Kieffer, 1997). In terms of intron-exon organization, the coding sequence for the ORL1 receptor is nearly identical to those of the μ-, δ- and κ-opioid receptors, suggesting that the four genes have evolved from a common ancestor, hence they belong to the same family.

2.2. Localization of mRNA and protein

Hybridohistochemical detection in murine brain and cord sections have localized ORL1 transcripts in many discrete areas, particularly the cortical and cortico-limbic areas (amygdala, hippocampus, habenula, septum), hypothalamus (ventromedial and paraventricular nuclei), brain stem (locus coeruleus, parabrachial nucleus, central gray, dorsal raphe nucleus) and the dorsal and ventral horns of the spinal cord (Bunzow et al., 1994; Fukuda et al., 1994; Mollereau et al., 1994; Wick et al., 1994; Lachowicz et al., 1995). ORL1 receptor mRNA is also present in cell bodies of the dorsal root ganglion (Wick et al., 1994). None of

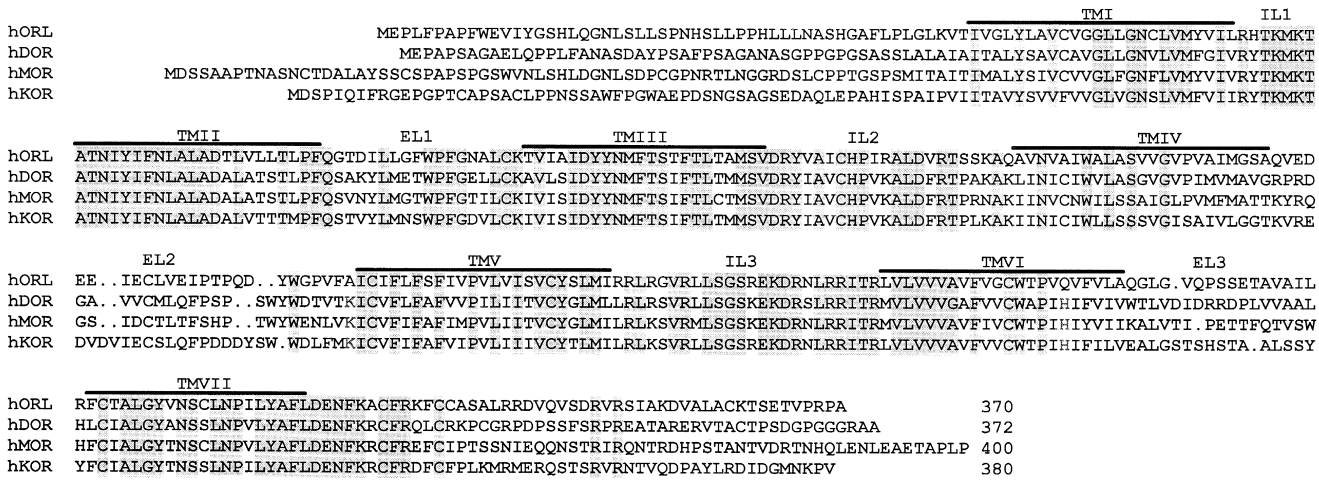

Fig. 1. Comparison of amino acid sequences of human ORL1, δ (DOR), μ (MOR) and κ (KOR) opioid receptors, deduced from genomic sequences. Identical amino acid residues are shaded. TM: transmembrane domain, IL: intracellular loop, EL: extracellular loop.

these studies revealed detectable expression of the ORL1 gene in the caudate-putamen and cerebellum. Extensive immunohistochemical mapping of the ORL1 protein in the central nervous system of the rat, using a monoclonal antibody to the N-terminus of the receptor, yielded distributions in agreement with those obtained from in situ hybridization studies, indicating that the ORL1 receptor is predominantly expressed in local-circuit (inter)neurones (Anton et al., 1996). After noc/oFQ was identified (see below, Section 3.1.), the elegant approach of agonist-induced incorporation of [^{35}S]GTPγS in brain slices (Sim et al., 1995) was applied successfully in the rat to map the ORL1 receptor to the cortex, amygdala, hypothalamus, thalamus and brain stem (Sim et al., 1996). The wide distribution and localization of the ORL1 receptor mRNA and/or protein, suggested the receptor has the potential to modulate a variety of central processes, including learning and memory, attention and emotions, movement and motor processes, homeostasis, neuroendocrine secretion, and virtually every modality of sensory perception (visual, auditory, olfactory, gustatory, somaesthetic and nociceptive).

Northern blot analysis has not been particularly successful in revealing ORL1 transcripts outside of the central nervous system. However, using the more sensitive reverse transcriptase-polymerase chain reaction assay, Wang et al. (1994) report the presence of ORL1 receptor mRNA in rat intestine, skeletal muscle, vas deferens and spleen. The ORL1 receptor message is also present in several subpopulations of mouse lymphocytes (Halford et al., 1995), as well as human lymphocytes and lymphocytic cell lines (Wick et al., 1995), implicating a role of the ORL1 receptor in immune function.

2.3. Binding properties in vitro

In spite of its close similarity to opioid receptors, the ORL1 receptor binds opioids and antagonists with considerably lower affinity than opioid receptors do, such that binding of opioids to ORL1 could only be demonstrated indirectly. In recombinant chinese hamster ovary (CHO) cells expressing the ORL1 receptor, etorphine was found to inhibit forskolin-induced accumulation of cAMP, but at doses (ED_{50} = 0.7 μM) three orders of magnitude higher than those required to produce the effect via opioid receptors (Mollereau et al., 1994). Recently however, another opiate, lofentanil was shown to be 100-fold more potent than etorphine (7 vs 700 nM) in the ORL1 receptor-mediated cyclase inhibition assay, although interestingly, fentanyl was inactive (Butour et al., 1997). Furthermore, dynorphin A and dynorphin A-(1–13) stimulate K^+ currents in *Xenopus* oocytes co-injected with the cRNAs to the XOR1 receptor and a G protein-activated K^+ channel, suggesting that dynorphins are endogenous ligands for the orphan receptor (Zhang and Yu, 1995). Together, these data showed that the ORL1 receptor had partially retained opioid receptor characteristics, a notion that has been confirmed by the observation that very few point mutations suffice to confer the ORL1 receptor with opioid (κ) receptor binding characteristics (Meng et al., 1996; Mollereau et al., 1996a).

3. Nociceptin/orphanin FQ, the endogenous ligand of the ORL1 receptor

3.1. Identification

Based upon the structural analogy of ORL1 and κ-opioid receptors, the endogenous ligand of the ORL1 receptor was expected to be a dynorphin-like peptide. It was, therefore, sought after in acetic acid extracts from nerve tissue as a basic peptide which would inhibit adenylyl cyclase in recombinant CHO cells expressing the receptor. Fractionation of rat brain and porcine hypothalamus ex-

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beta-endorphin        YGGFMTSEKSQTPLVTLFKNAIIKNVHKKGQ
Met-enkephalin        YGGFM
Leu-enkephalin        YGGFL
alpha-neoendorphin    YGGFLRKYPK
noc/oFQ               FGGFTGARKSARKLANQ
dynorphin A           YGGFLRRIRPKLKWDNQ
                      message_________
                               address
```

Fig. 2. Comparison of amino acid sequences of noc/oFQ and a few endogenous opioid peptides. Identical amino acid residues are shaded. The 'message' and 'address' domains of dynorphin A, as defined by Chavkin and Goldstein (1981), are indicated.

tracts by conventional procedures ultimately led two laboratories (Meunier et al., 1995; Reinscheid et al., 1995) to independently isolate and identify a heptadecapeptide whose primary structure is indeed similar to that of dynorphin A (Fig. 2). Initially, the synthetic peptide was shown to strongly inhibit forskolin-induced accumulation of cAMP in CHO cells expressing the ORL1/LC132 receptor (ED_{50} at about 1 nM) and, when injected intracerebroventricularly in mice, to induce hyperalgesia in the hot plate (Meunier et al., 1995) and tail-flick (Reinscheid et al., 1995) tests. Meunier et al. termed the novel peptide 'nociceptin' (noc), after its apparent pro-nociceptive properties, while Reinscheid et al. named it 'orphanin FQ' (oFQ), the ligand of an orphan receptor, whose first and last amino acids are Phe (F) and Gln (Q), respectively. The peptide was later identified as the endogenous ligand of the mouse ROR-C receptor and reported to induce allodynia following intrathecal administration in conscious mice (Okuda-Ashitaka et al., 1996).

3.2. Precursor cDNA and gene

Evidence that the novel peptide must be physiologically relevant and synthesized locally was provided by the isolation, from a rat brain library, of a (partial) cDNA encoding a larger precursor polypeptide whose sequence contains one copy of noc/oFQ, framed by Lys-Arg proteolytic excision motifs (Meunier et al., 1995). Interestingly, the full-length cDNA was being independently isolated not as the cDNA encoding the peptide's precursor, but as an orphan cDNA whose mRNA (*N23K*) is transiently up-regulated during dibutyryl-cAMP-induced neurite outgrowth of mouse neuroblastoma NS20Y cells (Saito et al., 1995). The primary structure of the mouse and rat noc/oFQ precursors was later confirmed (Houtani et al., 1996; Nothacker et al., 1996; Pan et al., 1996). A variant form of precursor cDNA, *N27K*, has been identified in dibutyryl cAMP-treated NS20Y cells. It encodes a protein of 212 amino acids, in which the two last amino acids in the shorter form are replaced by a stretch of 27 amino acids (Saito et al., 1996).

The prepronociceptin/orphanin FQ (ppnoc/oFQ) gene has been partially characterized (Mollereau et al., 1996b). It consists of at least four exons interspaced by three introns. Exon I contains exclusively 5′ noncoding sequence, exons II and III share the open reading frame of the gene, and exon IV contains most of the 3′ noncoding region of the message. The nucleotide sequence of the murine and human ppnoc/oFQ genes displays organizational and structural features that are very similar to those of the genes encoding the precursors to the endogenous opioid peptides, prepro-enkephalin, -dynorphin and -opiomelanocortin, suggesting that the nociceptin and opioid peptide genes have evolved in parallel from a common ancestor gene. The ppnoc/oFQ gene has been mapped to the short arm of human chromosome 8 (8p21), close to the locus encoding the neurofilament light chain EFL (Mollereau et al., 1996b).

The amino acid sequence of the noc/oFQ precursor is highly conserved across murine and human species (Fig. 3), especially the C-terminal third which hosts noc/oFQ itself. The lowest sequence identity is observed in the core of the molecule where the acidic motif (DAEPGA) is repeated in the murine, but not the human precursor. The noc/oFQ precursor contains a putative N-terminal signal peptide of about 20 amino acids and, interestingly, other putative cleavage sites that do not flank the noc/oFQ

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mouse   mkilfcdvlllsllssvfssCPRDCLTCQEKLHPAPDSFNLKTCILQCEEKVFPRPLWTVCTKVMASGSGQLSPADPELV
rat     mkilfcdvlllsllssvfssCPEDCLTCQERLHPAPGSFNLKLCILQCEEKVFPRPLWTLCTKAMASDSEQLSPADPELT
human   mkvllcdllllslfssvfssCQRDCLTCQEKLHPALDSFDLEVCLIECEEKVFPSPLWTPCTKVMARSSWQLSPAAPEHV
        ** * ** ***** *******  ******* ****  ** *  *** ******* **** *** **  * ***** **

mouse   SAALYQPKASEMQHLKRMPRVRSLVQVLDAEPGADAEPGADAEPGADDAEEVEQKQLQKRFGGFTGARKSARKLANQKRF
rat     SAALYQSKASEMQHLKRMPRVRSVVQARDAEPEA------DAEPVADEADEVEQKQLQKRFGGFTGARKSARKLANQKRF
human   AAALYQPRASEMQHLRRMPRVRSLFQEQEE-PEP----------GMEEAGEMEQKQLQKRFGGFTGARKSARKLANQKRF
         ***** ******** *******  *     *                * * ****************************

                                  NV                                    187
mouse   SEFMRQYLVLSMQSSQRRRTLHQNG
                                  IQVIPRTACVHSKTCRPGVRIPPSPRH           212 (N27K)
rat     SEFMRQYLVLSMQSSQRRRTLHQNGNV                                     181
human   SEFMRQYLVLSMQSSQRRRTLHQNGNV                                     176
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Fig. 3. Comparison of amino acid sequences of mouse, rat and human noc/oFQ precursors, deduced from genomic sequences. The sequence of the putative signal peptide is indicated in lower case letters. Asterisks: conserved amino acid residues. Overlined: conserved Cys residues. Underlined: putative processing cleavage sites. The noc/oFQ peptide sequence is shaded. The sequence of the N27K variant of the mouse precursor (Saito et al., 1996) is also shown.

sequence (Fig. 3). One, Lys-Arg in mouse and rat, and Arg-Arg in human, is located upstream of noc/oFQ in the precursor sequence, and the other, Arg-Arg-Arg, downstream. This raises the possibility that ppnoc/oFQ may be the precursor not only to noc/oFQ, but also to other bioactive neuropeptides, in particular the C-terminal peptide of 28 amino acids whose sequence is strictly conserved across murine and human species, and/or its further processing products, the heptadecapeptide FSEFMRQYLVLSMQSSQ and the octapeptide TLHQNGNV. No clear biological activity has yet been reported for these peptides. Nor is there, at present, any evidence that they have specific receptors.

3.3. Localization

Northern blot analyses have shown the ppnoc/oFQ gene to be predominantly expressed, as a major mRNA species $\approx$ 1.3 kb long, in the brain and spinal cord of rat and human (Mollereau et al., 1996b; Nothacker et al., 1996; Pan et al., 1996). In the human brain, expression levels are high in amygdala and subthalamic nuclei, intermediate in hypothalamus, substantia nigra, and thalamus, and low in corpus callosum and hippocampus (Nothacker et al., 1996). The 1.3 kb mRNA species has also been detected in rat ovary (Mollereau et al., 1996b), human spleen, leukocytes and fetal, but not adult, kidney (Nothacker et al., 1996). Also present in human spleen and fetal brain mRNA preparations, is a larger species (5 kb) that may correspond to a nonspliced primary transcript (Nothacker et al., 1996). In the mouse brain, expression of the N27K mRNA encoding the longer form of ppnoc/oFQ (see above), is developmentally regulated, and occurs maximally soon after birth (Saito et al., 1996).

In situ hybridization and immunocytochemical studies have provided more detailed information of where the ppnoc/oFQ gene (but not necessarily the encoded noc/oFQ product) is expressed. Houtani et al. (1996) observe hybridization signals that are, (i) most abundant in the central gray, central tegmental field, nucleus of the lateral lemniscus, superior olive and spinal trigeminal nucleus of the brainstem, (ii) less so in cortical layers I–III, lateral septum, nucleus of the lateral olfactory tract and bed nucleus of the stria terminalis, lateral geniculate nucleus and the medial preoptic, ventromedial and supramammillary hypothalamic nuclei, pontine nuclei, inferior colliculus, pontomedullary raphe nucleus and nucleus of the lateral lemniscus, and (iii) sparse in many other areas, including the olfactory bulb and CA1–3 hippocampal regions. Within the spinal cord, ppnoc/oFQ transcripts are abundant in laminae I–III of the dorsal horn and lamina X, but are virtually absent in the caudate-putamen and cerebellum. The N27K protein has been identified immunochemically in neurone subsets throughout the central and peripheral nervous systems, including cell bodies of the paraventricular and arcuate nuclei of the hypothalamus, the dorsal and ventral horns of the spinal cord, and small- but not large-sized neurones of the dorsal root ganglion (Saito et al., 1996).

Noc/oFQ has also been mapped using specific antibodies to the peptide. In the rat spinal cord, noc/oFQ immunoreactivity (ir) is particularly robust in the superficial dorsal horn, lateral spinal nucleus and the region dorsal to the central canal (Riedl et al., 1996). High levels of noc/oFQ in the dorsal, but not the ventral horn have also been found by Schuligoi et al. (1997), who used a quantitative radioimmunoassay. Noc/oFQ-ir resists unilateral dorsal rhizotomy, indicating that the peptide originates from central, rather than primary afferent neurones (Riedl et al., 1996). In the brain, noc/oFQ-ir is detected in the sensory trigeminal complex, raphe nuclei, locus coeruleus, periaqueductal grey, amygdala, hypothalamic and septal areas (Schulz et al., 1996). Although many of these brain and cord regions also contain opioid peptides, confocal microscopic analysis of two-colour double-stained sections reveals only rare occurrences of co-localization of noc/oFQ and opioid peptides in nerve fibres and terminals (Riedl et al., 1996; Schulz et al., 1996).

Overall, the distribution of pro-noc/oFQ transcripts and/or of the noc/oFQ peptide in the central nervous system is clearly distinct from that of the precursors to opioid peptides and/or the peptides themselves, and qualitatively matches the distribution of the ORL1 receptor mRNA.

3.4. Binding properties and structure-activity relationships

In membrane preparations derived from CHO cells expressing the ORL1 receptor, ^{125}I-labeled [Tyr^{14}]noc/oFQ, which is equipotent with noc/oFQ in the ORL1 receptor-mediated cyclase inhibition assay (Reinscheid et al., 1995), and [^{3}H]noc/oFQ (Butour et al., 1997; Mollereau et al., 1996b) bind a single class of sites with very high affinity ($K_D \approx 0.1$ nM). In rat brain membranes (Dooley and Hougthen, 1996), or in membranes from NG 108-15 neuroblastoma $\times$ glioma hybrid cells (Ma et al., 1997), substantially higher K_D values (3–5 nM) have been reported, possibly reflecting different experimental (ionic) conditions. Some evidence for noc/oFQ receptor heterogeneity in mouse brain homogenates has been brought recently, using [^{125}I-Tyr^{14}]noc/oFQ (Mathis et al., 1997). Equilibrium binding of [^{3}H]noc/oFQ to the ORL1 receptor appears to be regulated (inhibited) by Na^+ ions and/or guanyl-5′-yl imidodiphosphate (Butour et al., 1997), in the same way as binding of opioids to opioid receptors (Francés et al., 1985).

The primary structure of noc/oFQ displays structural similarities with dynorphin A (Fig. 2); it was initially suggested that the two neuropeptides have a similar functional architecture (Meunier et al., 1995). Dynorphin A has been proposed to comprise two domains, a ‘message’ domain (the *N*-terminal tetrapeptide Tyr-Gly-Gly-Phe) re-

sponsible for biological (opioid) activity, and an 'address' domain (Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys) that confers the peptide enhanced potency and/or receptor selectivity (Chavkin and Goldstein, 1981). Indeed, compared to noc/oFQ, des-Phe^1-noc/oFQ displays considerably impaired ability to bind and/or activate the ORL1 receptor (Dooley and Hougthen, 1996; Butour et al., 1997; Matthes et al., 1996; Yu et al., 1997). Nevertheless, a Phe residue in position 1 of noc/oFQ is not as stringent a requirement for affinity and/or biological activity as is the Tyr residue in dynorphin A: [Tyr^1]noc/oFQ and noc/oFQ are equally potent when acting on the ORL1 receptor (Butour et al., 1997; Matthes et al., 1996; Mollereau et al., 1996a; Reinscheid et al., 1996; Shimohigashi et al., 1996), whereas [Phe^1]dynorphin is much less active than dynorphin A towards opioid receptors (Walker, 1982). Alanine-scanning mutagenesis of noc/oFQ (Dooley et al., 1996; Reinscheid et al., 1996) has identified other amino acid residues critical for noc/oFQ activity, in particular Gly^2, Phe^4 and Arg^8. Most significantly, however, analysis of a series of C-terminally truncated noc/oFQ fragments (Butour et al., 1997; Dooley et al., 1996; Reinscheid et al., 1996; Shimohigashi et al., 1996) has revealed that the shortest, fully active fragment of noc/oFQ, noc/oFQ-(1–12 or 1–13) is somewhat longer than the shortest, fully active fragment of dynorphin A, dynorphin A-(1–7) (Chavkin and Goldstein, 1981). Furthermore, N-terminally truncated fragments, particularly noc/oFQ-(6–17), have been reported to display significant affinity for, and activity towards the ORL1 receptor, suggesting that the basic core of noc/oFQ may be a major determinant of biological activity (Butour et al., 1997). Indeed, screening of combinatorial peptide libraries has led Dooley et al. (1996) to identify highly basic hexapeptides (such as Ac-RYYRWK-NH_2) with nanomolar potency at the ORL1 receptor.

Although the functional architecture of noc/oFQ is not fully understood, it is probably different from that of dynorphin A. A recent nuclear magnetic resonance study has shown that the N-terminal part of noc/oFQ has the same conformational preferences as the message of dynorphin A, whereas the C-terminal sequence is more flexible than the corresponding address of the opioid peptide (Salvadori et al., 1997). In fact, a 'rhegnylogic' (vs 'sychnologic', see Schwyzer, 1977) organization of noc/oFQ (vs dynorphin A) might underly the observed differences. In any event, noc/oFQ and dynorphin A are not expected to bind their respective receptors at equivalent sites, congruent with the finding that an ORL1/κ-opioid hybrid receptor binds both noc/oFQ and dynorphin A with very high affinity (Mollereau et al., 1996c).

3.5. Noc/oFQ-induced early postreceptor events

Because the putative intracellular loops of the ORL1 and opioid receptors share high sequence identity (see Fig. 1), it was correctly anticipated that ORL1 couples with the same G protein-regulated cellular effectors as do opioid receptors, namely adenylyl cyclase, inwardly rectifying K^+ channels, and voltage-gated Ca^{2+} channels.

3.5.1. Adenylyl cyclase

High doses of the potent opiate etorphine ($ED_{50} = 0.7$ μM) or of the κ-opioid peptides dynorphin-(1–17) and -(1–13) (5 μM) were first reported to inhibit forskolin-induced accumulation of cAMP in recombinant CHO (Mollereau et al., 1994) or human embryonic kydney 293 (Zhang and Yu, 1995) cells stably expressing the ORL1/XOR1 receptor. Noc/oFQ was later shown to be a potent agonist of the ORL1/LC132 receptor in the same assay, with ED_{50}s around 1 nM (Meunier et al., 1995; Reinscheid et al., 1995). More recently, Ma et al. (1997) showed that the neuroblastoma $\times$ glioma NG 108-15 hybrid cell line expresses the ORL1 receptor, and that noc/oFQ inhibits adenylyl cyclase ($ED_{50} = 0.7$ nM) in these cells. This effect, which is blocked by pretreatment with *B. pertussis* toxin but is insensitive to naltrindole, undergoes homologous desensitization upon pre-challenge with the peptide. Inhibition of forskolin-induced accumulation of cAMP by noc/oFQ has also been reported in mouse brain homogenates ($ED_{50} \leq 1$ nM; Mathis et al., 1997).

3.5.2. Inwardly-rectifying K^+ conductance

Dynorphin A and dynorphin A-(1–13) induce an inward K^+ current in *Xenopus laevis* oocytes injected with cRNAs to the XOR1/ORL1 receptor and a G protein-activated K^+ channel, with ED_{50} values of 45 and 37 nM, respectively (Zhang and Yu, 1995). Using a similar approach, Matthes et al. (1996) find that nanomolar concentrations of noc/oFQ half-maximally stimulate potassium influx. In rat brain slices containing the dorsal raphe nucleus, noc/oFQ increases ($ED_{50} = 12$ nM) the inwardly rectifying K^+ conductance. This effect is not reversed in the presence of 1 μM naloxone (Vaughan and Christie, 1996). Likewise, noc/oFQ increases ($ED_{50} = 90$ nM) the same inwardly rectifying K^+ conductance as do μ-opioid, somatostatin and α_2-adrenergic receptor agonists in rat brain slices containing the locus coeruleus (Connor et al., 1996b). The peptide action is subject to rapid desensitization ($t_{1/2} = 120$ s). Whole-cell patch-clamp recording ex vivo has also demonstrated noc/oFQ stimulation of outward K^+ currents in rat periaqueductal gray neurones (Vaughan et al., 1997). Together, these data are consistent with the hypothesis that noc/oFQ acts primarily to reduce synaptic transmission in the nervous system.

3.5.3. Voltage-gated Ca^{2+} channels

SH-SY5Y human neuroblastoma cells express the ORL1 receptor naturally (Cheng et al., 1995). In these cells, noc/oFQ produces a concentration-dependent, albeit partial (30–40%), inhibition of an N-type Ca^{2+} conductance, with an IC_{50} value of about 40 nM (Connor et al., 1996a).

Interestingly, noc/oFQ also mobilizes intracellular Ca^{2+}, but only in the presence of concurrent stimulation with carbachol. Both effects are abolished upon pretreatment of the cells with *B. pertussis* toxin and are insensitive to the opioid receptor antagonists D-Phe-Cys-Tyr-D-Trp-Arg-Pen-Thr-NH_2 (CTAP), naltrindole and naloxone. In dissociated rat hippocampal neurones, the three major types of Ca^{2+} channel, L, N and P/Q are partially inhibited in the presence of noc/oFQ, but at substantially higher doses than those required to inhibit adenylyl cyclase in recombinant non-neuronal cells, or to stimulate K^+ currents in brain slices (see above). Inhibition by the peptide is no longer seen after *B. pertussis* treatment and cannot be prevented by high doses of naloxone (Knoflach et al., 1996). It is noteworthy that the N-type current, which is primarily involved in the control of exocytosis of fast-acting transmitters, is the most sensitive to the peptide.

The actions of noc/oFQ on ion conductances are expected to reduce neuronal excitability and/or presynaptic transmitter secretion. There is indeed direct evidence that it is so. Noc/oFQ has been reported to suppress the K^+-evoked release of glutamate from rat cerebrocortical slices (Nicol et al., 1996), the light-evoked release of acetylcholine from cholinergic (presumably amacrine) neurones in the rabbit eye-cup preparation (Neal et al., 1997), and the basal and electrically-induced release of enkephalin in the guinea pig myenteric plexus (Gintzler et al., 1997). There is also indirect evidence that noc/oFQ depresses glutamatergic transmission in the spinal cord (Faber et al., 1996), and exerts presynaptic inhibitory actions both on excitatory glutamatergic and inhibitory GABAergic transmission in rat periaqueductal gray neurones (Vaughan et al., 1997). Similarily, Giuliani and Maggi (1996) find that noc/oFQ inhibits the release of tachykinin in the guinea pig renal pelvis preparation.

3.6. Metabolism

Biotransformation of noc/oFQ in human blood has been monitored by MALDI mass spectrometry (Yu et al., 1996). Although no half-life times for the peptide were reported, it could be inferred from the data shown that noc/oFQ is relatively stable in whole blood, the main biotransformation product being des-Phe^1-noc/oFQ. Hence, intravenous injection might be appropriate to probe for peripheral actions of the peptide.

In the presence of mouse brain cortical slices noc/oFQ is predominantly cleaved at bonds Phe^1-Gly^2, Ala^7-Arg^8, Ala^{11}-Arg^{12} and Arg^{12}-Lys^{13}, in the main by aminopeptidase N and endopeptidase 24.15 (Montiel et al., 1997). Endopeptidase 24.11 ('enkephalinase') does not seem to be involved. From a practical perspective, it is noteworthy that degradation of noc/oFQ in contact of brain slices can be almost totally prevented in the presence of 100 μM EDTA. These studies have also pointed to the substantially greater resistance of noc/oFQ to degradation than dynorphin A or the enkephalins. Involvement of aminopeptidase N and endopeptidase 24.15 in degradation of noc/oFQ is confirmed in vivo by the fact that i.c.v. co-administration of inhibitors of the two enzymes will potentiate the motor-depressant action of the peptide in the rat (Noble and Roques, 1997).

4. Central effects of noc/oFQ in vivo (summarized in Table 1)

4.1. Pain and analgesia

Based on the distribution of its transcripts in brain and spinal cord sections, and before its endogenous ligand was identified, the ORL1 receptor had been unanimously considered to play a role in nociperception. Along this line, repeated i.c.v. injection into mice of an oligonucleotide complementary -antisense- to the ORL1 receptor mRNA, results in significant hypoalgesia, as assessed in the hot plate test (Meunier et al., 1995). The corresponding human oligonucleotide sequence is inactive, indicating that the action is a specific one. However, mice lacking the ORL1 receptor have been found to display the same nociceptive thresholds as control mice, both in the tail-flick and writhing tests (Nishi et al., 1997). When first administered intracerebroventricularly in mice, noc/oFQ (0.05 nmol/animal) was reported to increase reactivity to a painful stimulus (hyperalgesia) in the hot plate assay (Meunier et al., 1995). Noc/oFQ-induced hyperalgesia in mice was also observed in the mouse tail-flick test, following i.c.v. injection of 1–10 nmol (Reinscheid et al., 1995; Rossi et al., 1996; Nishi et al., 1997), or of as low as 0.5 pmol (Shimohigashi et al., 1996) of peptide. This effect is no longer apparent in ORL1 receptor-deficient homozygous mice (Nishi et al., 1997). Modest, although significant hyperreactivity to electric foot shock has also been reported following intrathecal administration of noc/oFQ (20 nmol) in the pregnant rat and in animals under hormone-stimulated pregnancy status (Dawson-Basoa and Gintzler, 1997). In an invertebrate, the land snail *Cepaea nemoralis*, Kavaliers and Perrot-Sinal (1996) show that systemically administered noc/oFQ (5 pmol) elicits clear hyperreactivity to aversive thermal stimulation while dynorphin A, at the same dose, induces the opposite (analgesia). The latter effect, but not the former, is reversed by the κ-opioid receptor antagonist nor-binaltorphimine. Finally, intrathecal administration of ≥ 0.5 pmol noc/oFQ induces *allodynia*, a pain response to non-noxious (tactile) stimulation, in mice (Okuda-Ashitaka et al., 1996).

The notion that noc/oFQ possesses intrinsic pronociceptive (hyperalgesic) activity, especially in the mouse, was soon reassessed by Mogil et al. (1996b), on the basis that the animals generally used as controls, i.e. saline i.c.v.-injected mice, must be under stress-induced analge-

Table 1
Published in vivo central effects of nociceptin/orphanin FQ

	Animal species	Test	Route of administration	Dose (nmol)[a]	Effect	Reference
Pain and analgesia						
Nociperception	mouse	hot plate	i.c.v.	0.005–0.05	hyperalgesia[b]	Meunier et al. (1995)
	-	tail-flick	-	1–10	hyperalgesia[b]	Reinscheid et al. (1995)
	-	-	-	5	hyperalgesia[b]	Rossi et al. (1996)
	-	-	-	0.0005–0.5	hyperalgesia[b]	Shimohigashi et al. (1996)
	-	-	-	10	hyperalgesia[b]	Nishi et al. (1997)
	-	writhing	-	2.5	no effect[c]	Mogil et al. (1996b)
	-	hot plate	-	2.5	no effect[c]	-
	-	tail withdrawal	-	1–10	no effect[c]	-
	rat	electric foot shock	i.t.	20	hyperalgesia	Dawson-Basoa and Gintzler (1997)
	snail	hot plate	systemic	0.00005–0.005	hyperalgesia	Kavaliers and Perrot-Sinal (1996)
	mouse	light touch	i.t.	0.0005–0.25	allodynia	Okuda-Ashitaka et al. (1996)
	mouse	tail-fick	i.c.v.	5	analgesia[d]	Rossi et al. (1996)
	rat	-	i.t.	0.5–5	analgesia	Xu et al. (1996)
	mouse	-	-	10	analgesia[d]	King et al. (1997)
	rat	-	-	3–10	analgesia	Tian et al. (1997a)
Hyperalgesia induced by:						
Nerve constriction	rat	paw withdrawal	-	17	inhibition	Yamamoto et al. (1997a)
Carageenan injection		-	-	-	inhibition	Yamamoto et al. (1997b)
Analgesia induced by:						
Stress	mouse	writhing	i.c.v.	2.5	inhibition	Mogil et al. (1996b)
	-	hot plate	-	2.5	-	-
	-	tail withdrawal	-	1–10	-	-
Opioids	mouse	tail withdrawal	i.c.v.	10	inhibition	Mogil et al. (1996a)
	-	-	i.t.	25	no effect	Grisel et al. (1996)
	-	tail flick	i.c.v.	0.0004–10	inhibition	Tian et al. (1997a)
	-	-	i.t.	0.1–10	potentiation	-
Electroacupuncture	rat	tail flick	i.c.v.	0.1–10	inhibition	Tian et al. (1997b)
Gestation	-	electric footshock	i.t.	1–10	inhibition	Dawson-Basoa and Gintzler (1997)
Locomotor activity	mouse	activity box	i.c.v.	10	decrease	Reinscheid et al. (1995)
	-	-	-	0.005–0.05	increase	Florin et al. (1996)
	-	-	i.c.v.	1–10	decrease	Noble and Roques (1997)
	-	-	-	10	decrease	Nishi et al. (1997)
	rat	-	-	10–100	decrease	Devine et al. (1996a)
	-	-	-	1–10	decrease	Devine et al. (1996b)
	-	-	-	10	decrease	Sandin et al. (1997)
	-	-	i.t.	0.5–5	no effect	Xu et al. (1996)
	-	-	-	3–10	no effect	Tian et al. (1997a)
Learning	rat	place navigation	hippocampus	10	suppression	Sandin et al. (1997)
Motivation	rat	place preference	i.c.v.	0.1–100	no effect	Devine et al. (1996b)
	-	DA release in N.acc.		15–150	suppression	Murphy et al. (1996)
Feeding	rat	food intake	i.c.v.	1–10	increase[d]	Pomonis et al. (1996)
	-	-	N. acc., VMH	2.5–25	increase	Stratford et al. (1997)
Hormone secretion						
Basal	rat	growth hormone	i.c.v.	1–6	decrease	Bluet-Pajot et al. (1997)
Stress-induced	-	prolactin and ACTH	-	6	potentiation	-

[a] Rounded values using 2,000 as the molar mass of noc/oFQ; [b] vs saline i.c.v.-injected animals; [c] vs uninjected animals; [d] reversed by naloxone.
I.c.v.: intracerebroventricular; i.t.: intrathecal; N. acc.: nucleus accumbens; VMH: ventromedial hypothlamic nucleus.

sia. In three different assays, the mouse writhing, hot plate and tail withdrawal assays, these authors show convincingly that noc/oFQ-injected (0.5–10 nmol, i.c.v.) and uninjected animals are equally sensitive to noxious stimulation while, in contrast, vehicle-injected animals display a decreased nociceptive sensitivity. Indeed, rather than inducing hyperalgesia, noc/oFQ appears to reverse a naloxone-sensitive, stress (needle prick)-induced analgesia, lead-

ing Mogil and collaborators to conclude that noc/oFQ is a functional anti-opioid peptide. Not only does i.c.v. administered noc/oFQ reverse stress-induced analgesia, but also antinociception by morphine given systemically (Mogil et al., 1996b) and the opioid receptor type-selective agonists [D-Ala^2, MePhe^4, Gly-ol^5]enkephalin (DAMGO, μ); [D-Thr^2, D-Leu^5]enkephalin (DTLET, δ), and trans,3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclo-hexyl]-benzeneacetamide (U-50488H, κ), given intracerebroventricularly (Mogil et al., 1996a). Noc/oFQ was entirely ineffective in reversing analgesia induced by morphine given intrathecally, indicating that the peptide acts as a supraspinal, but not a spinal anti-opioid peptide (Grisel et al., 1996; Tian et al., 1997a). However, opioid-mediated elevation of nociceptive thresholds during physiological or hormone-stimulated pregnancy in the rat, are reversed upon spinal administration of 1–10 nmol noc/oFQ (Dawson-Basoa and Gintzler, 1997). Finally, noc/oFQ administered intracerebroventricularly (vs intrathecally) reverses (vs potentiates) electroacupuncture-induced analgesia (EAIA) in the rat, as assessed in the tail-flick test. Interestingly, antisense nucleotides to the ORL1 receptor mRNA enhance EAIA in the rat, suggestive of a tonic antagonism of EAIA by endogenous noc/oFQ (Tian et al., 1997b).

Noc/oFQ also appears to be endowed with *spinal antinociceptive* properties. In the mouse tail-flick test, noc/oFQ (5 nmol, i.c.v.) produces an initial hyperalgesia followed by an analgesia which is completely blocked by naloxone, indicative of an (indirect) opioid mechanism of action (Rossi et al., 1996; King et al., 1997). In the rat tail-flick test, intrathecally-administered noc/oFQ (0.5 and 5 nmol) induces robust and long-lasting ($\geq$ 60 min at the higher dose) analgesia (Xu et al., 1996), and there is no cross-tolerance between the antinociceptive effects of i.t. noc/oFQ and morphine, indicating that the two substances act via distinct receptors (Hao et al., 1997). Likewise, in the decerebrated, spinal rat preparation, i.t. noc/oFQ depresses a spinal nociceptive flexor reflex, even in the presence of opioid, $GABA_A$ or α_2-adrenergic receptor antagonists (Xu et al., 1996). Tian et al. (1997a) too, report that i.t. noc/oFQ induces spinal analgesia in the rat (tail-flick test), although the effect is modest and of short duration ($\leq$ 10 min), and is clearly observed only at the fairly high dose of 10 nmol peptide. In the same test, these authors also find that i.t. noc/oFQ potentiates analgesia by morphine administered subcutaneously. Electrophysiological studies in the cord have also provided indirect evidence that noc/oFQ behaves as a spinal analgesic. For example, noc/oFQ (25 and 112.5 nmol, i.t.) inhibits the C-fibre evoked wind-up and post-discharge of dorsal horn neurones, an effect that is reversed by 50 μg, but not 10 μg naloxone (Stanfa et al., 1996). In keeping with these results are the recently published observations that in the rat, noc/oFQ (17 nmol, i.t.) inhibits thermal hyperalgesia (spinal facilitation) induced by chronic unilateral constriction of the sciatic nerve (Yamamoto et al., 1997a), and carageenan injection into the paw (Yamamoto et al., 1997b). Likewise, in the neonatal rat hemisected spinal cord preparation, noc/oFQ depresses both the A and C fibre-mediated components of the glutamatergic population ventral root potential elicited by electrical stimulation of the dorsal root. In this study, noc/oFQ is found to be most effective (ED_{50} = 30 nM) in depressing the C fibre-mediated component. These effects are insensitive to 1 μM naloxone (Faber et al., 1996). Finally, microiontophoretically applied noc/oFQ inhibits the NMDA- and natural stimuli-evoked responses of neurones in the superficial and deeper dorsal horn of the medulla (trigeminal nucleus caudalis) in the rat (Wang et al., 1996). Interestingly, the peptide's inhibitory action is not modality specific: nociceptive, wide dynamic range and low threshold (non-nociceptive) neurones respond equally well, suggesting that modulation by noc/oFQ of neuronal activity in the cord is not restricted to nociperception alone. Here again, the peptide's inhibitory actions were not prevented in the presence of naloxone.

4.2. Locomotion

When injected intracerebroventricularly into mice, 10 nmol noc/oFQ causes a decrease in horizontal and vertical (rearing) locomotor activities, and induces ataxia and loss of the righting reflex (Reinscheid et al., 1995). Similar observations have been reported by Noble and Roques (1997), using similar doses of noc/oFQ and the same route of injection. Again in mice, noc/oFQ has been observed to stimulate, rather than inhibit, locomotor activiy and exploratory behaviour, however at comparatively lower (5–50 pmol) i.c.v. doses (Florin et al., 1996). The latter effects are insensitive to naloxone yet they are reversed by dopamine D_1 and D_2 receptor antagonists, suggesting that they are supported by increased dopamine transmission. In the rat, pronounced and long-lasting motor impairment, including disruption of coordination, balance and muscle tone is induced upon i.c.v. injection of 10 and 100 nmol noc/oFQ (Devine et al., 1996a; see also Stratford et al., 1997). Tolerance to these effects develops rapidly (within 2 or 3 days) and remains apparent 7 days after the last noc/oFQ injection (Devine et al., 1996a). When injected directly into the hippocampus, noc/oFQ (10 nmol) does not affect motor performance in the rat, but the peptide reduces markedly the short-lasting (exploratory) increase in locomotion and rearing activity displayed in response to novelty (Sandin et al., 1997). The hypolocomotor action of noc/oFQ appears to be specifically supraspinal since rats which have received 0.5–10 nmol of peptide intrathecally do not show any sign of motor deficit (Xu et al., 1996; Tian et al., 1997a). Finally, there does not seem to be any tonic noc/oFQ regulation of locomotion in mice since homozygous mutant mice lacking the ORL1 receptor gene display no obvious abnormality in spontaneous and/or exploratory locomotor behaviour (Nishi et al., 1997).

In the rat, the motor effects of noc/oFQ differ from those of μ- and δ-opioid receptor agonists, that are known to stimulate locomotion, but they are reminiscent of the non-opioid motor impairment caused by i.c.v.- administered dynorphin A (references in Devine et al., 1996a,b).

4.3. Motivation

The mesolimbic dopamine pathway, which projects from the ventral tegmental area to the nucleus accumbens, is a common target for motivationally active drugs (see Le Moal and Simon, 1991, and Koob, 1992, for reviews). Positive reinforcers, such as μ-opioid receptor agonists, will stimulate this pathway, and negative ones, such as κ-opioid receptor agonists, inhibit it (Spanagel et al., 1992, and references therein). Interestingly, Murphy et al. (1996) report that, in the anaesthetized rat, noc/oFQ (15–150 nmol/animal, i.c.v.) suppresses dopamine release in the nucleus accumbens, an indication that the peptide ought to be endowed with aversive properties. However, Devine et al. (1996b) find that noc/oFQ (0.1–100 nmol/animal, i.c.v.) is motivationally neutral in the rat place preference/aversion test.

Furthermore, in contrast to what one might have expected given the peptide's supraspinal anti-opioid properties, and abundance in the locus coeruleus, noc/oFQ (2, 10 or 50 nmol) does not precipitate a withdrawal syndrome when injected into the lateral ventricle in the morphine-dependent rat (Tian et al., 1997a). However, other sites of injection need to be explored, since anti-opioids such as neuropeptide FF, will precipitate a vigorous abstinence syndrome when injected into the third, but not the lateral ventricle in morphine-treated rats (reviewed by Panula et al., 1996).

4.4. Cognitive processes

The high levels of the ORL1 message (Mollereau et al., 1994) and protein (Anton et al., 1996) in hippocampus called for an involvement of noc/oFQ in learning and memory processes. Indeed, local microinjection into this region of 10 nmol noc/oFQ dramatically impairs spatial learning in the rat, as assessed in the place navigation test (Sandin et al., 1997). A similar effect is observed following injection of nanomole amounts of dynorphins into the CA3 region of the hippocampus (Sandin et al., submitted). Indirect evidence that noc/oFQ may play a role in cognitive processes is also provided by electrophysiological data in the CA1 and dentate regions of rat hippocampal slices, where bath-applied noc/oFQ (μM) reduces both the slope of excitatory postsynaptic potentials and population spike amplitude, and inhibits induction of long-term potentiation at the Schaffer collateral-CA1 synapse (Yu et al., 1997). Along the same lines, Knoflach et al. (1996) report that noc/oFQ partially inhibits voltage-gated Ca^{2+} channels in hippocampal CA1 and CA3 neurones, and Ikeda et al. (1997) show that, in mouse hippocampal slices, bath-applied noc/oFQ (μM) induces hyperpolarization, via inward-rectifier K^+ channels, of CA3 pyramidal neurones, suggesting that the peptide may modulate long-term potentiation at the mossy fibre-CA3 synapse.

4.5. Feeding

Just like opioids do, especially κ-opioid receptor agonists (reviewed by Morley et al., 1983), noc/oFQ stimulate food intake in the rat. In one study (Pomonis et al., 1996), it is shown that i.c.v.-injected noc/oFQ (1, 3 and 10 nmol) induces feeding in satiated animals and that this effect is completely blocked by s.c. administration of naloxone (1 mg/kg). In the other (Stratford et al., 1997), noc/oFQ (2.5–25 nmol) is administered intracranially, into the shell of nucleus accumbens or ventromedial hypothalamic nucleus, and is found in both cases to elicit a clear, however short-lasting orexigenic effect.

4.6. Neuroendocrine secretion

Preliminary results indicate that noc/oFQ regulates secretion of pituitary hormones, as could be anticipated from the presence of peptide and ORL1 receptor in various hypothalamic nuclei. In the rat, noc/oFQ (2.5 and 12.5 μg, i.c.v.) increases the basal level of circulating growth hormone, but not of prolactin and corticotropin. The higher dose of peptide partially reverses the decrease in growth hormone, and enhances the increase in prolactin and corticotropin elicited in response to ether stress in the animals (Bluet-Pajot et al., 1997). Whether or not these effects are anti-opioid in nature and ORL1 receptor-mediated remains to be determined.

4.7. Neuronal differentiation

In mouse NS20Y neuroblastoma cells, dibutyryl cAMP induces neurite outgrowth and concomitant up-regulation of the N23K and N27K noc/oFQ precursors (Saito et al., 1995, 1996). Transient up-regulation of the N23K and N27K mRNA and protein is also observed in the developing mouse brain, expression being highest in the early postnatal brain. Interestingly, the N27K protein appears to accumulate in the tips of neurites in NS20Y cells undergoing dibutyryl cAMP-induced neurite outgrowth and, in the developing brain, to be present in virtually all neuroblasts, and to redistribute according to known patterns of neuroblast migration (Saito et al., 1996). Although it is not known whether it is the N27K protein or its putative maturation products (in particular noc/oFQ) that are physiologically important, these findings suggest that the N27K protein must be involved, in some way, in brain development.

5. Peripheral effects of nociceptin/orphanin FQ (summarized in Table 2)

5.1. Smooth muscle

Several ex vivo and in vivo studies have now identified noc/oFQ activities in peripheral tissues, especially smooth

Table 2
Published in vivo and ex vivo peripheral effects of nociceptin/orphanin FQ

	Animal species	Test	Route of administration	Dose (nmol)[a]	Effect	Reference
Heart and vessels						
Heart rate	rat		i.c.v.	0.5–15	decrease	Kapusta et al. (1997)
	-		i.v.	10/kg/min	no effect	-
Mean arterial pressure	-		i.c.v.	0.5–15	decrease	-
	-		i.v.	10/kg/min	decrease	-
	-		i.v.	3–30/kg	decrease	Champion and Kadowitz (1997)
Urinary tract						
Urinary flow rate	rat		i.c.v.	0.5–15	increase	Kapusta et al. (1997)
	-		i.v.	10/kg/min	increase	-
Urinary Na^+ excretion	-		i.c.v.	0.5–15	decrease	-
	-		i.v.	10/kg/min	decrease	-
Isolated organs						
Renal pelvis	guinea pig	electrically induced	bath	28 nM[b]	inhibition	Giuliani and Maggi (1996)
Vas deferens	mouse	contraction	-	20 nM[b]	inhibition	Berzetei-Gurske et al. (1996)
	-	-	-	8 nM[b]	inhibition	Calo et al. (1996)
	rat	-	-	50 nM[b]	inhibition	Berzetei-Gurske et al. (1996)
	rabbit	-	-	1 μm	no effect	Calo et al. (1996)
Ileum	guinea pig	-	-	13 nM[b]	inhibition	-
Arterial rings:						
carotid	cat	phenylephrine-induced		0.1–100 nM	relaxation	Gumusel et al. (1997)
mesenteric	-	contraction	-	-	relaxation	-
renal	-	-	-	-	relaxation	-
femoral	-	-	-	-	relaxation	-

[a]Rounded values using 2,000 as the molecular mass of noc/oFQ; [b]1C50 values.

muscle. Noc/oFQ ($ED_{50} \approx 30$ nM) inhibits the inotropic response to electrical field stimulation of the guinea pig isolated renal pelvis, an effect which may reflect suppression of tachykinin release from peripheral sensory nerve endings (Giuliani and Maggi, 1996). The peptide inhibits the electrically-induced contractions of the mouse vas deferens with an IC_{50} value of ≈ 20 nM, and an amplitude of $\approx 65\%$ of the maximal attainable inhibition (Berzetei-Gurske et al., 1996). Calo et al. (1996) report similar values ($ED_{50} = 10$ nM, 76% maximum inhibition) and, in addition, find that the guinea pig ileum is responsive to nociceptin (pIC_{50}: 8.12, maximum inhibition: 50%). Noc/oFQ is also a potent (ED_{50} in the nanomolar range) vasorelaxant on phenylephrine pre-contracted rings from cat renal, mesenteric, carotid and femoral arteries (Gumusel et al., 1997). In vivo, the peptide appears to be active when administerd by the intravenous route. In rats, continuous infusion (10 nmol/kg/min; Kapusta et al., 1997) or bolus injection (30 nmol/kg; Champion and Kadowitz, 1997) of noc/oFQ cause a decrease in mean arterial pressure.

5.2. Kidney

In addition, intravenous infusion of noc/oFQ (10 nmol/kg/min) markedly increases urine flow whilst suppressing urinary sodium excretion in the rat (Kapusta et al., 1997). Similar effects are observed upon i.c.v. administration of the peptide (1, 10 and 30 μg/animal). In the same study, noc/oFQ and dynorphin A (10 μg/animal, i.c.v.) are both shown to induce diuresis and concurrent antinatriuresis, although the action of the opioid peptide is prevented by nor-binaltorphimine whereas the action of noc/oFQ is not. The observation that noc/oFQ produces similar effects whether administered peripherally or centrally raises the possibility that the peptide has the ability to cross the blood–brain barrier.

5.3. Immune cells

Finally, although the peptide has not yet been tested directly, noc/oFQ may play a role in immune function. ORL1 mRNA transcripts are present in both stimulated and unstimulated mouse splenic lymphocytes (Halford et al., 1995), human peripheral blood lymphocytes and the human T-leukemic HSB-2, CEM-3 and MOLT-4 and Raji Burkitt's lymphoma cell lines (Wick et al., 1995). The ORL1/AT7-5EU message is enhanced at least 10-fold upon treatment of human blood lymphocytes with phytohemagglutinin (ibid), and ORL1/oOR-specific antisense nucleotides will inhibit lipopolysaccharide-induced mouse lymphocyte proliferation and production of immunoglobulins G and M (Halford et al., 1995). Taken together, these data strongly suggest that the receptor is involved in the regulation of immunocompetence. However, Nishi et al. (1997) fail to detect abnormalities in immunoglobulin

content of peripheral blood or in populations of lymphocytes in blood and bone marrow, of ORL1 receptor-deficient mice.

6. Summary and perspectives

Homology cloning strategies (see for instance Libert et al., 1989) and more recently the sequencing of whole genomes, have identified many open reading frames encoding proteins of unknown function, in particular putative G protein-coupled membrane receptors (Parmentier et al., 1995). Identification of orphan receptors in this way has marked the advent of 'reverse pharmacology' to identify the corresponding physiological ligands (Mills and Duggan, 1993; Schwartz, 1993). This approach has proven of great heuristic value and has led to the discovery of the ORL1 receptor and its natural ligand, nociceptin/orphanin FQ, the basic components of a new peptide-based signalling pathway in the nervous sytem.

Based on genetic criteria, there is little doubt that the ORL1 and opioid receptors belong to the same family. On functional grounds however, the ORL1 receptor is not an opioid receptor: it does not bind opioid receptor ligands with high affinity and most importantly, the pharmacological effects it mediates are insensitive to opioid receptor antagonists. Likewise, there is little doubt that noc/oFQ and opioid peptides are homologous. Yet, noc/oFQ is not an opioid peptide: it does not bind to opioid receptors with high affinity, and the pharmacological effects it elicits are insensitive to opioid receptor antagonists. The marked structural analogy between the ORL1 and opioid receptors, especially the κ-opioid receptor, and the noc/oFQ and opioid peptides, particularly dynorphin A, is, however, not reflected anatomically since noc/oFQ and opioid peptides appear to be located in separate neuronal circuits. As far as can be judged, noc/oFQ triggers the same G protein-mediated signalling pathways as do opioids and other neuromodulatory agents in order to elicit a physiological response. In particular, by stimulating an outward K^+ current and/or inhibiting voltage-gated Ca^{2+} channels, noc/oFQ reduces synaptic efficacy, i.e. neuronal activity. Nevertheless, it is clear that inhibition by noc/oFQ of inhibitory neurones can also result in enhanced neuronal activity, dependent upon the circuitry. Indeed, it is known that morphine excites dopamine mesocorticolimbic neurones indirectly, by relieving a tonic, GABA-mediated inhibition (Di Chiara and North, 1992, and references therein).

It is, therefore, not surprising that the in vivo pharmacological profile of noc/oFQ differs in several respects from, and even opposes, those of opioids. In spite of sometimes conflicting data, the overall trend, in the rat, is for noc/oFQ to act supraspinally thus inhibiting opioid-mediated analgesia, while convergent electrophysiological and behavioural data indicate the peptide ought to be considered a spinal analgesic (Section 4.1.). Similar actions have been reported for anti-opioid peptides, in particular neuropeptide FF (reviewed in Panula et al., 1996). Yet, unlike neuropeptide FF, noc/oFQ has not yet been found to precipitate withdrawal in morphine-tolerant rats. Nor does noc/oFQ elicit motivational effects (Section 4.3.), suggesting the peptide lacks abuse liability. Again, by acting supraspinally, noc/oFQ impairs motor performance (Section 4.2.), suppresses spatial learning (Section 4.4.), induces feeding (Section 4.5.), and regulates basal and stress-induced release of pituitary hormones (Section 4.6.). It is worthy of note that noc/oFQ is also active when administered intravenously, a property which has been taken advantage of to demonstrate its potent smooth muscle relaxant (Section 5.1.), diuretic, and antinatriuretic (Section 5.2.) properties. Last but not least, noc/oFQ appears to regulate stimulated immune function (Section 5.3.), and to be involved in neuronal differentiation (Section 4.7.). Although these multiple pharmacological actions of noc/oFQ have not been validated by the use of a selective ORL1 receptor antagonist, since one is not yet available, great care has generally been taken to verify that they are not prevented by opioid receptor antagonists.

Although the discovery of noc/oFQ, a neuropeptide with multiple functions, does not introduce new concepts in fundamental brain mechanisms, it will certainly improve our knowledge of brain physiology, and may find therapeutical applications, for example in the management of pain or hyponatremic and water-retaining diseases. However, given the wide distribution of noc/oFQ and its receptor, the pharmacological profile of noc/oFQ is likely to be incomplete, and other as yet unknown functions of the peptide remain to be discovered. Most helpful in this respect will be the identification of new ligands of the ORL1 receptor, particularly antagonists. There are many approaches to this important goal (Petsko, 1996, and accompanying articles). Such ligands may even already exist in the vaults of the pharmaceutical industry. Others await the process of rational design, perhaps based on the structure of non-peptide ligands of the ORL1 receptor, such as the opiate lofentanil (Butour et al., 1997) and/or the σ ligands carbetapentane and rimcazole, that act as antagonists of the ORL1 receptor (Kobayashi et al., 1997). If research on noc/oFQ carries on unabated at the present pace, potentially clinically interesting new compounds could become available in the not too distant future.

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