Structure-Activity Relationship Studies on the Novel Neuropeptide Orphanin FQ*

(Rceived for publication, February 21, 1996, and in revised form, April 4, 1996)

Rainer K. Reinscheid, Ali Ardati, Frederick J. Monsma, Jr., and Olivier Civelli†
From CNS Research, Pharma Division, F. Hoffmann-La Roche AG, CH-4070 Basel, Switzerland

The heptadecapeptide orphanin FQ (OFQ) is an endogenous ligand to an opioid-like G protein-coupled receptor. Although the primary structure of OFQ exhibits some similarity to the opioid peptides, OFQ is not recognized by opioid receptors nor does the OFQ receptor bind opioid ligands. In order to investigate the structural determinants of this ligand/receptor selectivity, we conducted a systematic structure-activity study on OFQ to characterize which sites of the molecule are important for receptor activation. Alanine- and D-amino acid-scanning mutagenesis revealed several residues in the amino-terminal half of OFQ which participate in both receptor binding and activation. Most strikingly, the Phe1 position could be changed to a tyrosine without loss of biological activity. In addition, the OFQ receptor seemed to require recognition of the complete peptide molecule for activation. These results indicate that the mode of interaction of OFQ with its receptor may be different from that of the opioid peptides with their respective receptors and might therefore account for the observed selectivity.

We have recently isolated a novel neuropeptide from porcine brain, named “orphanin FQ” (OFQ),† that acts as an endogenous ligand to a particular G protein-coupled receptor (1). Similar results were reported independently by another group who called the same peptide “nociceptin” (2). Its corresponding receptor (formerly named LC132 or ORL-1, but also see references in Ref. 1) had been discovered in several laboratories and exhibited a significant degree of sequence identity with the cloned opioid receptors (~60%); however, it did not bind any of the known opiate ligands (3, 4). The primary structure of OFQ shares several sequence motifs with the opioid peptides that are known to be important for biological activity of the opioid peptides. In particular, the amino-terminal tetrapeptide Phe-Gly-Gly-Phe in OFQ is strikingly similar to the canonical sequence Tyr-Gly-Gly-Phe found in all natural mammalian opioid peptides. However, OFQ did not show any activity at the opioid receptors (1). This high level of receptor/ligand specificity is in contrast to the well-known promiscuity in receptor activation of the opioid peptides (5, 6).

It has been postulated that the opioid peptides primarily interact with a common binding site on their receptors via the amino-terminal pentapeptide moiety (Tyr-Gly-Gly-Phe-Met/Leu in all mammalian species), whereas the carboxyl-terminal extensions are believed to determine their relative selectivities toward a specific subtype of opioid receptor. The notion of such a bipartite structure contained within the same molecule fostered the development of the so-called “message-address concept” (7, 8). According to this theory, the “message” domain consists of the amino-terminal Tyr-Gly-Gly-Phe motif that can also serve as the minimally active structure and unequivocally defines such peptides as “with opiate-like activity.” The carboxyl-terminal extensions of the opioid peptides are supposed to contain the “address” part which determines their individual receptor selectivity without exerting biological activity on their own.

The absence of interaction between OFQ and the opioid receptors and vice versa could indicate that OFQ interacts with its receptor via a mechanism different from that of the opioids with their receptors. In order to determine the structural components important for OFQ to activate its receptor, we investigated the pharmacological activity of a series of OFQ analogs. We also examined the contribution of each amino acid in OFQ to support a biologically active conformation of the peptide. Our data suggest that, in contrast to the opioid system, the OFQ receptor might recognize different parts of the OFQ molecule and seems to require the complete peptide structure for biological activity, thus leading to the observed selectivity.

EXPERIMENTAL PROCEDURES

Peptides and Chemicals—Orphanin FQ and all analogs were synthesized by Research Genetics (Huntsville, AL). Opioid peptides were from Bachem (Bubendorf, Switzerland), and non-peptidergic agonists and antagonists were purchased from RBI (Natick, MA). 125I-Labeled [Tyr14]orphanin and cAMP radiimunoassay kits were obtained from Amersham Corp. Forskolin was purchased from Calbiochem, and HPLC columns and solvents were from Merck (Darmstadt, FRG). All other chemicals were of analytical grade and purchased from Merck, Fluka, or Sigma. Tissue culture reagents were obtained from Life Technologies, Inc.

Transfected Cell Lines and Culture Conditions—The OFQ receptor was cloned and stably expressed in CHO cells as described previously (1). Stable clones of HEK 293 cells expressing the OFQ receptor were obtained after transfection of the corresponding cDNA doped into the mammalian expression vector pRcRSV (Invitrogen, San Diego, CA) using a calcium phosphate precipitation protocol (9). Colonies were selected with 418 (Life Technologies, Inc.) and screened for expression by reverse transcriptase-polymerase chain reaction and functional assays. Cells were cultured at 5% CO2 in α-modified Eagle’s medium containing 5% fetal calf serum and 500 μg/ml G418.

Synthesis of Mono- and Diiodinated [Tyr14]Orphanin—Mono- and diiodinated [Tyr14]orphanin were synthesized by the chloramine-T method and purified by HPLC (10). Briefly, 10 μl of 0.1 mm [Tyr14]orphanin in 0.5 mM sodium phosphate buffer, pH 7.5, was mixed with an equal volume of 0.1 m NaI in 0.1 mM phosphate buffer, pH 7.5. The reaction was initiated by adding 10 μl of a chloramine-T solution (1 mmol in 0.1 mM phosphate buffer, pH 7.5). After 30 s, 500 μl of Na2SO3 (3 mmol in deionized water) was added, and the reaction mixture was loaded onto a LiChrosphere RP select B HPLC column (125 × 3 mm) equilibrated with 11% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. Reaction products were eluted with a linear gradient to 35% acetonitrile in 0.1% trifluoroacetic acid over 30 min while the
effluent was monitored at 215 nm. Individual peaks were collected manually. The identity of the mono- and diiodinated species was confirmed by matrix-assisted laser desorption mass spectrometry.

Receptor Binding and Competition Experiments—Ligand binding assays were done in 96-well deep bottom microwell plates. CHO cells stably expressing the OFQ receptor were harvested and resuspended in 10 volumes of binding buffer containing 50 mM Tris-HCl, pH 7.8, 5 mM MgCl₂, 1 mM EGTA, 0.1% bovine serum albumin, and 0.1 mg/ml each of aprotinin, leupeptin, and pepstatin. Membranes were prepared using a tissue homogenizer (setting 4 for 30 s; PT20, Kinematica, Lucerne, Switzerland). Total membrane particulate was obtained after centrifugation at 45,000 × g for 10 min at 4 °C. The resulting pellet was resuspended and centrifuged twice as described above. Membranes were finally diluted in an appropriate volume of binding buffer and stored at −70 °C. Protein concentration was determined using a BCA Protein Assay Kit (Pierce). Incubation of membranes (10–18 mg of protein) with [125I-Tyr14]orphanin was performed at room temperature for 60 min in a total volume of 200 μl of binding buffer. For competition binding experiments 50,000 cpm of [125I-Tyr14]orphanin (62.5 pm) were added together with the indicated concentrations of unlabeled peptides. Non-specific binding was determined in the presence of 50 nM orphanin FQ. Bound and free ligand were separated by rapid vacuum filtration through Whatman GF/C filters using a Brandel multichannel harvester. GF/C filters had been pretreated with 0.3% polyethylenimine containing 0.1% bovine serum albumin for 1 h at room temperature. Filters were washed six times with 1 ml of ice-cold 50 mM Tris-HCl, pH 7.5, and then counted in a gamma counter. All experiments were done at least three times in triplicate. Binding data were analyzed with the LIGAND software (11), and curves were fitted with the Kaleidagraph software.

Measurement of cAMP Accumulation in Receptor-transfected Cells—Adenylyl cyclase assays were carried out as described previously (1). L-Alanine and d-amino acid-substituted orphanin peptides were initially tested at a single concentration of 1 μM. Some peptides were investigated in more detail to compare dose-response data with receptor binding data. For the investigation of potential antagonistic properties of OFQ analogs, peptides (all at 1 μM) were coadministered with 10 nM OFQ. To elucidate the involvement of G protein coupling in intracellular signal transduction, OFQ receptor-transfected CHO cells were plated in 24-well clusters and preincubated with 10 ng/ml pertussis toxin in culture medium for 18 h before testing. All experiments were done at least three times in triplicate. Dose-response curves were fitted and calculated with a Kaleidograph.

RESULTS

Demonstration of G Protein Involvement in Signal Transduction—The effect of OFQ on forskolin-stimulated cAMP accumulation in OFQ receptor-transfected cells was completely abolished after pretreatment with 10 ng/ml pertussis toxin (Fig. 1). These results indicate that the intracellular transduction of receptor activation might be mediated by a G,i-like protein.

Development of a Radioligand for the OFQ Receptor—In order to develop an analog of OFQ that would be suitable for radiodination, we synthesized a series of tyrosine-substituted peptides and compared their biological activity with that of the authentic OFQ (for sequences see Table I). OFQ inhibited forskolin-stimulated adenylyl cyclase activity in CHO and HEK 293 cells expressing the OFQ receptor with half-maximal effective concentrations (EC₅₀) of 1.05 ± 0.28 and 0.98 ± 0.11 nM, respectively (Fig. 1 and Table II).

The exchange of tyrosine for Phe² or Leu¹⁴, respectively, did not affect biological activity as measured in cAMP assays, whereas the substitution of Phe² dramatically reduced the ability to inhibit forskolin-stimulated adenylyl cyclase activity (Fig. 1 and Table II). Since the amino-terminal domain of the opioid peptides is known to play a crucial role for their biological activity, we decided to use [Tyr¹⁴]orphanin as a substrate for radiodination. In a “cold” labeling experiment we first synthesized both the mono- and diiodinated species, separated them on HPLC, and confirmed their identity by mass spectrometry ((M + H) = 1952 for [monoiodo-Tyr¹⁴]orphanin and (M + H) = 2078 for [diiodo-Tyr¹⁴]orphanin; data not shown). Both peptides were tested in cAMP assays. [monoiodo-Tyr¹⁴]orphanin retained full biological activity with an EC₅₀ of 1.32 ± 0.79 nM, whereas the dose-response curve of the diiodinated analog was slightly shifted to the right showing an EC₅₀ of 1.78 ± 0.5 nM (Fig. 1 and Table II). Consequently, [¹²⁵I-Tyr¹⁴]orphanin was used as a radioligand in binding studies.

Ligand Binding Studies at the OFQ Receptor—[¹²⁵I-Tyr¹⁴]orphanin bound to membranes prepared from CHO cells expressing the OFQ receptor in a saturable and displaceable manner with a Kᵣ of 56.2 ± 7.3 μM and a Bₘₐₓ of about 200 fmol/mg protein (data not shown). The Hill coefficient was close to unity in all experiments. Although [¹²⁵I-Tyr¹⁴]orphanin is supposed to be an agonist at the OFQ receptor, we did not observe more than one affinity constant using the LIGAND program.

OFQ inhibited the binding of [¹²⁵I-Tyr¹⁴]orphanin to the OFQ receptor with an inhibitory constant (Kᵣ) of 0.19 ± 0.02 nM (Fig. 2 and Table II). The [¹²⁵I-Tyr¹⁴]orphanin analogs also showed high potencies in inhibiting [¹²⁵I-Tyr¹⁴]orphanin binding, with Kᵣ values of 0.13 ± 0.02 and 0.68 ± 0.06 nM.

![Fig. 1. Effect of OFQ and related peptides on forskolin-stimulated cAMP levels in CHO cells stably expressing the OFQ receptor. The involvement of G proteins in signal transduction was investigated after pretreatment of the cells with pertussis toxin. Data were normalized to the amount of cAMP in forskolin-stimulated cells (100%). Incubations were done at least two times in triplicate. Average values of a representative experiment are shown. □, orphanin FQ; ▪, [Tyr¹⁴]orphanin; ○, [Tyr¹⁴]orphanin; ●, [Tyr¹⁴]orphanin; ▲, [Tyr¹⁴]orphanin; △, [Tyr¹⁴]orphanin; ○, orphanin FQ on cells treated with 10 ng/ml pertussis toxin for 18 h.](http://www.jbc.org/)

| Table I | Orphanin FQ and related peptides |
|---------|----------------------------------|
| **Name** | **Sequence**                     |
| Orphanin FQ | Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln |
| [Tyr¹⁴]orphanin | Tyr-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln |
| [Tyr¹⁴]orphanin | Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln |
| Orphanin (1–11) | Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln |
| Orphanin (1–7) | Phe-Gly-Gly-Phe-Thr-Gly-Ala |
| Orphanin (1–5) | Phe-Gly-Gly-Phe-Thr |
| Orphanin (10–17) | Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln |
Structure-Activity Relationships of Orphanin FQ

Comparison of biological activities and competition binding potencies of orphanin FQ and related peptides

Data are given as average ± S.D. of mean where applicable and were calculated from at least three independent experiments done in triplicate. ND, not determined.

| Peptides          | Inhibition of forskolin-stimulated cAMP, EC50 (± S.E.) | Receptor binding, Kd (± S.E.) |
|-------------------|-----------------------------------------------------|-----------------------------|
| Orphanin FQ (CHO) | 1.05 (±0.28)                                        | 0.19 (±0.02)                |
| Orphanin FQ (HEK 293) | 0.98 (±0.11)                                    | ND                          |
| [Tyr1]Orphanin     | 1.03 (±0.67)                                        | 0.13 (±0.02)                |
| [Tyr1]Orphanin     | 242.7 (±77.3)                                       | 1.67 (±0.25)                |
| [Tyr1]Orphanin     | 1.02 (±0.11)                                        | 0.63 (±0.06)                |
| [Tyr1]Orphanin     | 1.32 (±0.79)                                        | 0.51 (±0.07)                |
| [Tyr1]Orphanin     | 0.18 (±0.16)                                        | 1.96 (±0.18)                |
| Orphanin-(1–11)    | >10,000                                             | 138 ± 22.0                  |
| Orphanin-(1–7)     | >10,000                                             | >1000                       |
| Orphanin-(1–5)     | >10,000                                             | >1000                       |
| Orphanin-(10–17)   | >10,000                                             | >1000                       |
| Dynorphin A        | >10,000                                             | 449 ± 70                    |
| Alanine scan peptides |                                                |                             |
| [Ala1]Orphanin     | >10,000                                             | 20.3 ± 6.8                  |
| [Ala1]Orphanin     | >10,000                                             | 18.2 ± 3.4                  |
| [Ala1]Orphanin     | 41.1 (±3.6)                                         | 0.09 (±0.02)                |
| [Ala1]Orphanin     | >10,000                                             | 36.9 ± 1.6                  |
| [Ala1]Orphanin     | >10,000                                             | 5.1 ± 2.3                   |
| [Ala1]Orphanin     | 142.1 (±11.4)                                       | ND                          |
| [Ser1]Orphanin     | 140.2 (±26.8)                                       | ND                          |
| [Ala1]Orphanin     | >10,000                                             | 18.1 ± 6.9                  |
| [Ala1]Orphanin     | 174.2 (±29.8)                                       | 1.17 ± 0.3                  |
| D-Ala scan peptides |                                                |                             |
| [D-Phe1]Orphanin   | 105.6 (±25.8)                                       | 2.30 (±0.35)                |
| [D-Ala1]Orphanin   | 4.74 (±1.32)                                        | 0.61 (±0.27)                |
| [D-Ala1]Orphanin   | >10,000                                             | 16.9 ± 2.5                  |
| [D-Phe1]Orphanin   | >1000                                               | 2.71 (±0.69)                |
| [D-Thr1]Orphanin   | >1000                                               | 14.37 (±5.7)                |
| [D-Ala1]Orphanin   | 100.6 (±22.2)                                       | ND                          |
| [D-Ala1]Orphanin   | 33.9 (±2.4)                                         | 0.14 (±0.04)                |
| [D-Arg1]Orphanin   | >1000                                               | 1.44 (±0.43)                |

D-Amino acid scan peptides

[●-Phe1]-, [●-Ala1]-, [●-Phe4]-, [●-Thr5]-, [●-Ala7]-, and [●-Arg1]-orphanin were either unable to inhibit forskolin-stimulated cAMP accumulation in receptor-transfected CHO cells or had a markedly reduced potency (Fig. 3). Again, the significant reduction in biological activity contrasted with their moderate reduction in binding affinity (Table II). Both structural and conformational changes of Arg2 yielded less active peptides, as compared with the authentic OFQ. In general, it was noticed that almost any alteration in the amino-terminal part of OFQ reduced the biological activity, whereas receptor binding potentials often did not reflect these differences.

All alanine and D-amino acid-substituted peptides were also examined for potential antagonistic potency, expressed as their ability to reverse the effect of 10 nM orphanin FQ on forskolin-stimulated adenyl cyclase activity. Only [●-Ala1]-orphanin displayed a weak antagonism. As shown in Fig. 4, 100 nM [●-Ala1]-orphanin coadministered with increasing concentra-
oralanine or D-amino acid substitutions. Alanine scanning of a variety of OFQ analogs, representing either truncated peptides or analogs of natural opioid peptides, was performed.

We have utilized a method to inhibit forskolin-stimulated cAMP accumulation in OFQ receptor-transfected CHO cells. All peptide analogs were applied at 1 µM, and data were normalized to cAMP levels in forskolin-stimulated cells (0%). For comparison, the effect of 1 µM OFQ and no treatment are shown at the top. Results represent the average ± S.E. of duplicate determinations from three independent experiments.

DISCUSSION

We have investigated the structural requirements for binding and receptor activation of orphanin FQ, a novel neuropeptide which is related to the opioid peptides. We have utilized a variety of OFQ analogs, representing either truncated peptides or alanine or D-amino acid substitutions. Alanine scanning mutagenesis of other bioactive peptides, like the enkephalins (12), dynorphin A (13), dermorphin (14), or bradykinin (15), has been shown useful in characterizing the contribution of the respective amino acid side chain to biological activity. Substitutions by D-amino acids introduce a conformational change in the peptide backbone and are therefore more suitable to detect the contribution of individual amino acids to the secondary structure of the peptide required for activity (16).

The results of these studies indicate distinct differences in the structural requirements for OFQ activity as compared with the opioid peptides. We found that position 1 in the OFQ primary structure does not stringently require a Phe residue, since substitution by tyrosine is tolerated without loss of biological activity or receptor affinity. Replacement by D-Phe results in a partially active peptide, whereas an alanine-substituted analog is inactive. Thus, the presence of an aromatic side chain in position 1 seems to be the minimal consensus. This is in striking contrast to the opioid peptides where any change of the Tyr position leads to a complete loss of activity (for an overview see Refs. 17, 18).

Introduction of L-alanine in place of Gly2 leads to an almost inactive compound, whereas substitution with the corresponding D-enantiomer does not affect biological activity or binding potency. Glycine is known to allow for conformational flexibility of peptide backbones. It is therefore likely that the more bulky side chain of L-alanine in this position prevents the formation of a favorable secondary structure, whereas D-alanine might rather mimic the conformation induced by the natural glycine residue. Similar results have been reported for the enkephalins where a substitution of Gly2 by L-alanine causes a profound loss of activity, but D-Gly2 analogs possess an even increased potency as compared with the natural peptide (17).

Structural or conformational changes in the Gly2 position lead to a decrease or loss of biological activity. While the [L-Ala3] analog still activated the receptor with an EC50 of ~40 nM, [D-Ala3]orphanin is inactive. Again, similar structural requirements have been demonstrated for the enkephalins at this position (17, 18).

Any change of the Phe residue in position 4 results in a complete loss of biological activity. Even the introduction of a hydroxyl moiety into the amino acid side chain (as in the case of [Tyr4]orphanin) is detrimental. Comparable observations were made in structure-activity relationship studies with the enkephalins (17, 18). Despite the functional inactivity of [Tyr4]orphanin, it still has an appreciably high receptor binding affinity. However, we could not detect any functional antagonism of [Tyr4]orphanin in cAMP assays. We are currently analyzing this discrepancy.

Changes of Thr5 cause a marked reduction of both biological activity and binding affinity. This is in some contrast to the opioid peptides where a greater structural freedom is already indicated by the natural occurrence of Leu or Met in this position. However, structure-activity relationship experiments with the enkephalins indicate that substitution of most other amino acids for Leu or Met also results in reduced binding affinity or biological potency (17).

In OFQ, Gly2 does not represent an absolute structural requirement for biological activity, since substitutions by L-Ala or
D-Ala result in only moderate losses of potency. Interestingly, substitution of Ala^7 by its D-enantiomer produces a partial agonist as determined by cAMP assays. The conformation of the peptide backbone at this position might therefore be necessary to attain or stabilize an agonistic higher order structure.

Replacement of Arg^8 by L-alanine results in a decrease of both functional potency and receptor affinity, whereas a conformational conversion in that position has a less profound effect. We therefore concluded that the positive charge of the arginine side chain, and to a lesser extent, its proper orientation are critical for receptor activation. The positive charge might actually interact with the side chain of a Glu or Asp residue on the receptor and thus serve to anchor the peptide. A similar yet less pronounced effect is observed for the Arg^12 position, where replacement by alanine or o-arginine reduces biological activity. However, both lysine residues in OFQ, whose side chains are also assumed to carry a positive charge under physiological conditions, can be substituted by alanine as well as D-Lys residues without loss of potency. Interestingly, for dynorphin A-(1–13) the importance of both arginine and lysine residues for biological activity has been demonstrated. Successive carboxyl-terminal deletions of dynorphin A-(1–13) identified Arg^7, Lys^11, and Lys^13 as contributing prominently to the peptide's high potency and opioid receptor selectivity (19), whereas conversion of all three arginine residues in dynorphin A-(1–9) to the corresponding D-enantiomers had only minor effects on receptor binding (16).

On the other hand, (Ala^8)dynorphin A-(1–13) retained only 35% of the dynorphin A-(1–13) receptor binding potency but showed an increased selectivity toward the k opioid receptor (20). In another study [Gln^8,Gly^31]human β-endorphin-Gly-Gly-NH2 (replacing the original Glu in position 8 by the uncharged Gln) was shown to be an effective antagonist, modulating analgesic responses to β-endorphin in vivo (21). These observations might indicate that a similar mode of receptor interaction, involving either the side chain of the amino acid in position 8 or the overall conformation influenced by this residue, could have been conserved among OFQ, dynorphin A, and β-endorphin. Whether this homology is dependent on a conserved structure within the corresponding receptors will be part of future studies using site-directed mutagenesis.

The exchange of all other amino acid residues in the carboxyl-terminal part of OFQ had little or no effect on their biological activity or receptor binding affinity. However, the overall structure of the carboxyl-terminal part of OFQ is indispensable, because all truncated peptides were devoid of any biological activity or reasonable affinity. Similar results have been obtained for β-endorphin, where removal of the last four amino acids leads to a complete loss of analgesic potency (21). On the other hand, it has been shown for dynorphin A that successive carboxyl-terminal truncation usually only results in a gradual decrease of biological potency and opioid receptor selectivity (19). However, we cannot exclude that truncated forms of OFQ, if generated in vivo, might interact with other hitherto unknown receptors.

Comparison of the data obtained for biological activity and receptor binding affinity of OFQ reveals some contradictions. In many cases it is not possible to detect receptor activation or antagonism, yet the peptides still exhibit appreciable affinities for receptor binding. For most of these peptides their K_i values are several hundredfold lower as compared with the natural OFQ (Table II). This observation may indicate the existence of a threshold binding affinity that must be surpassed for a biological effect to be elicited. The results of our alanine- and d-amino acid scanning mutagenesis indicate multiple amino acid residues critical for activation of the OFQ receptor. It is therefore possible that only the combined interaction of these residues with their binding sites on the receptor is able to induce an active conformation of the receptor. Such a model does not rule out binding of structurally altered peptides to some but not all of the binding sites on the receptor molecule. In fact, similar results have also been reported for a number of opioid peptide analogs (17, 18).

Taken together, these results demonstrate that in the aminoterminal part (e.g. positions 1–5) of the OFQ molecule, structural and conformational constraints for receptor activation are most stringent. This is quite similar to what has been shown for the opioid peptides, the striking difference being the greater structural freedom at position 1 of OFQ as compared with the opioid peptides and the importance of Thr^5 for receptor activation. In addition, the two arginine residues in position 8 and 12 seem to play an important role for biological activity of OFQ. Finally, there appears to be no minimally active structure present within the OFQ sequence, whereas the Leu- or Met-enkephalin moieties contained in all the opioid peptides can serve as full agonists, albeit with reduced potency and receptor selectivity. Our data imply that the complete OFQ molecule is necessary for biological activity, whereas the amino-terminal part extending up to position 8 contains most of the biological “information” for this effect. Such a bipartite structure resembles to some extent the message-address concept proposed for dynorphin A and the other opioid peptides (8, 19). In the case of the opioids this theory was used to explain the structural basis for receptor selectivity, based on the observation that a multitude of structurally similar opioid peptides could interact with any of the three main subtypes of opioid receptors, μ, δ, and κ, although with different efficiencies. Another central paradigm of this concept states that the message domain itself is sufficient to exert biological activity. Orphanin FQ does not interact with the opioid receptors and, conversely, the OFQ receptor does not bind opioid peptides. In addition, OFQ seems to lack a minimally active core. Our data, therefore, would not provide evidence for such a message domain within the OFQ molecule, so that by definition of the theory outlined above, OFQ can not be integrated into the message-address concept.

It will be interesting to investigate and identify the structural parts within both the opioid and the OFQ receptors responsible for the observed high degree of selectivity. Studies aimed at these questions might yield valuable information about the mechanisms determining receptor selectivity and could also help to explain the evolutionary events separating OFQ from the opioid peptide family.

Acknowledgment—We would like to thank Dr. H.-P. Nothacker for fruitful discussions and J. Higelin and R. A. Henningens for expert technical assistance.

REFERENCES

1. Reinscheid, R. K., Nothacker, H.-P., Boursen, A., Ardati, A., Henningens, R. A., Bunzow, J. R., Grandy, D. K., Langen, H., Monsma, F. J., Jr., and Civelli, O. (1999) Science 270, 792–794
2. Meunier, J.-C., Moliera, C., Toll, L., Suaudeau, C., Molsand, C., Alvinerie, P., Butzor, J.-L., Guillemin, J.-C., Ferrari, P., Monsarrat, B., Mazarguil, H., Vassart, G., Parmentier, M., and Costentin, J. (1995) Nature 377, 532–535
3. Bunzow, J. R., Saiz, C., Martruel, M., Bouvier, C., Williams, J. T., Low, M., and Grandy, D. K. (1994) FEBS Lett. 341, 284–288
4. Moliera, C., Parmentier, M., Mailleux, P., Butzor, J.-L., Molsand, C., Chalon, P., Caput, D., Vassart, G., and Meunier, J.-C. (1994) FEBS Lett. 341, 33–38
5. Goldstein, A., and Naidu, A. (1989) Mol. Pharmacol. 36, 265–272
6. Raynor, K., Kong, H., Chen, Y., Yasuda, K., Yu, L., Bell, G. I., and Reisine, T. (1993) Mol. Pharmacol. 45, 330–334
7. Schwyzer, R. (1977) Ann. N. Y. Acad. Sci. 297, 2–6
8. Goldstein, A. (1982) in Molecular Genetic Neuroscience (Schmidt, F. O., Bird, S. J., and Bloom, F. E., eds) pp. 249–262, Raven Press, Ltd., New York
9. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) in Molecular Cloning: A Laboratory Manual (Nolain C., ed) 2nd Ed., pp. 16.33–16.36, Cold Spring
10. Hunter, W. M., and Greenwood, F. C. (1962) Nature 194, 495–496
11. Munson, P. J., and Rodbart, D. (1980) Anal. Biochem. 107, 220–239
12. Beddell, C. R., Clark, R. B., Hardy, G. W., Lowe, L. A., Ubatuba, F. B., Vane, J. R., Wilkinson, S., Chang, K.-J., Cuatrecasas, P., and Miller, R. J. (1977) Proc. R. Soc. Lond. B Biol. Sci. 198, 249–265
13. Turcotte, A., Lalonde, J. M., Pierre, S. S., and Lemaire, S. (1984) Int. J. Pept. Protein Res. 23, 361–367
14. Darlak, K., Gronzka, Z., Krzascik, P., Janicki, P., and Gumulka, S. W. (1984) Peptides (Elmsford) 5, 687–689
15. Rudinger, J. (1971) in Drug Design (DeStevens, G., ed) Vol. 2, pp. 319–419, Academic Press, New York
16. Paterson, S. J., Kosterlitz, H. W., Vavrek, R. J., and Stewart, J. M. (1984) Neuropeptides 5, 177–180
17. Morley, J. S. (1980) Annu. Rev. Pharmacol. Toxicol. 20, 81–110
18. Hruby, V. J., and Gehrig, C. A. (1989) Med. Res. Rev. 9, 343–401
19. Chavkin, C., and Goldstein, A. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6543–6547
20. Lemaire, S., Lafrance, L., and Dumont, M. (1986) Int. J. Pept. Protein Res. 27, 300–305
21. Nicolas, P., Hammonds, R. G., Jr., and Li, C. H. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 3074–3077
Structure-Activity Relationship Studies on the Novel Neuropeptide Orphanin FQ
Rainer K. Reinscheid, Ali Ardati, Frederick J. Monsma, Jr. and Olivier Civelli

J. Biol. Chem. 1996, 271:14163-14168.
doi: 10.1074/jbc.271.24.14163

Access the most updated version of this article at http://www.jbc.org/content/271/24/14163

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 19 references, 5 of which can be accessed free at http://www.jbc.org/content/271/24/14163.full.html#ref-list-1