Characterization of Rat Prepro-Orphanin FQ/Nociceptin (154–181): Nociceptive Processing in Supraspinal Sites

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Received May 31, 2001; accepted August 13, 2001.

ABSTRACT
Orphanin FQ/nociceptin (OFQ/N), the endogenous ligand for the orphan receptor-like/x3-like opioid receptor clone, produces a variety of behavioral responses, including those associated with pronociception and antinociception. The OFQ/N precursor rattus-preorphanin FQ (rppOFQ/N) contains several paired basic amino acids, which raises the possibility that post-translational processing can be responsible for the production of a number of additional biologically active peptide fragments. One of these putative peptides, rppOFQ/N (rppOFQ/N154–181), was examined for antinociceptive and pronociceptive processes in four brain sites involved in pain inhibition: the ventrolateral periaqueductal gray (VLPG), the amygdala, the locus coeruleus (LC), and the rostroventromedial medulla (RVM). Endogenous rppOFQ/N154–181 was identified in each region. rppOFQ/N154–181 produced a dose-dependent antinociception in all four sites using the tailflick assay. Injections into misplaced cannula sites failed to exert effects. Antinociception in the four sites differed in their response to the opioid antagonist naloxone. Naloxone pretreatment completely blocked rppOFQ/N154–181-induced antinociception in the vlPAG and the amygdala, but not in the LC or RVM. In contrast rppOFQ/N154–181 was hyperalgesic in the LC and RVM, but not in the vlPAG or amygdala. rppOFQ/N154–181 also was compared with either its N-terminal 17-amino acid peptide (rppOFQ/N154–170, also known as OFQ2) or its 8-amino acid C-terminal fragment (rppOFQ/N154–161). Although both rppOFQ/N154–181 and rppOFQ/N154–170 produced antinociception, the latter was less effective because the C-terminal fragment was inactive. Thus, rppOFQ/N154–181 has complex antinociceptive and pronociceptive actions within the brain, and the pharmacological specificity of its actions differs among supraspinal sites.

Orphanin/FQ, or nociceptin (OFQ/N) (Meunier et al., 1995; Reinscheid et al., 1995), is generated from a larger proprotein, prepro-orphanin FQ/nociceptin (ppOFQ/N). Pharmacologically, the actions of OFQ/N are complex and appear to be contradictory (Grisel et al., 1996; Mogil et al., 1996; King et al., 1997; Rossi et al., 1997). OFQ/N is hyperalgesic when given alone (Meunier et al., 1995; Reinscheid et al., 1995; Rossi et al., 1996, 1997), and it can reverse opioid-induced antinociception (Grisel et al., 1996; Mogil et al., 1996; Tian et al., 1997). Furthermore, OFQ/N also has analgesic actions that become apparent when the hyperalgesic effects subside (Rossi et al., 1997). The spinal administration of OFQ/N has similar antinociceptive effects (King et al., 1997). Additionally, a related peptide (rppOFQ/N154–170) that is homologous in the mouse and rat was also found to elicit a similar antinociceptive response (Rossi et al., 1998).

Rat prepro OFQ/N (rppOFQ/N) contains several paired basic amino acids that can be proteolytically cleaved at multiple sites to potentially generate different peptides (Fig. 1). For instance, we have shown that a peptide corresponding to the full-length peptide rppOFQ/N154–181 is produced in the mouse hypothalamus (Mathis et al., 2001) and that administration of rppOFQ/N154–181 intracerebroventricularly (icv) produces potent antinociception in mice after supraspinal treatment (Mathis et al., 2001). The present studies explore the antinociceptive and pronociceptive effects of this new peptide, rppOFQ/N154–181 in four brain sites intimately involved in pain-inhibitory processes: the amygdala, the ventrolateral periaqueductal gray (vlPAG), the locus coeruleus.

ABBREVIATIONS: rppOFQ/N, rattus prepro-orphanin FQ/nociceptin; rppOFQ/N154–181, rattus prepro-orphanin FQ/nociceptin full-length peptide; OFQ2, prepro-orphanin FQ/N154–170 or beginning of full-length peptide; rppOFQ/N154–181, end of full-length peptide; vlPAG, ventrolateral periaqueductal gray; PAG, periaqueductal gray; LC, locus coeruleus; RVM, rostroventromedial medulla; icv, intracerebroventricularly; RP-HPLC, reverse phase-high-performance liquid chromatography.
**Materials and Methods**

**Cannulations**

Male Sprague-Dawley rats (275–500 g; Charles River Laboratories, Inc., Wilmington, MA) were housed individually, maintained on a 12-h light/dark cycle at ambient room temperature, and given food and water ad libitum. Each rat was anesthetized with chlorpromazine (3 mg/kg, i.p.; Sigma-Aldrich, St. Louis, MO) and ketamine (120 mg/kg, i.m.; Henry Schein, Port Washington, NY) prior to stereotaxic surgery. Rats were stereotaxically (Kopf Instruments, Tujunga, CA) implanted with one or more stainless steel guide cannulae (28 gauge; Plastics One, Roanoke, VA) aimed at either the amygdala, the vlPAG, the LC, the RVM, or the lateral ventricle (icv). For the amygdala cannulation, the incisor bar was set at −3 mm, and the coordinates were: 2.8 mm posterior to bregma, 3 mm lateral to the midline at an angle of 13° away from the sagittal suture, and 8.4 mm from the top of the skull. For the vlPAG cannulation, the incisor bar was set at −5 mm, and the coordinates were: 0.3 to 0.6 mm anterior to λ, 1.5 to 2.0 mm lateral to the sagittal suture at an angle of 12° toward the sagittal suture, and 6.8 to 7.0 mm from the top of the skull. LC coordinates were 1.5 mm posterior to the λ suture, 3.0 mm lateral to and toward the sagittal plane at a 15.5° angle, and 6.8 mm from the top of the skull. RVM coordinates were 10.8 to 11.3 mm posterior to the bregma suture, 0.0 to 0.7 mm lateral to the midline, and 9.5 to 11 mm from the top of the skull. Coordinates for the lateral ventricle cannula (22 gauge; Plastics One) were as follows: incisor bar was set at +5 mm, and the coordinates were: 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the sagittal suture, and 3.6 mm from the top of the skull. Cannulae were secured to the skull with three stainless steel screws and dental acrylic and kept patent with dummy cannulae during the weeks of testing. Each rat was allowed at least 1 week to recover before behavioral testing.

After completion of testing, all animals received an overdose of sodium pentobarbital (Euthanasia 0.5 ml; Henry Schein). Rats received a transcardiac perfusion with normal 0.9% saline followed by 10% buffered formalin. The brain was removed, blocked in 40-μm sections, mounted and stained with cresyl violet, and prepared for histological verification of cannula placements.

**Radiant Heat Tailflick Assay**

**Assay for Analgesia.** Rats were tested under baseline and experimental conditions in the radiant heat tailflick assay (IITC, Woodland Hills, CA) in which a high intensity thermal stimulus is delivered 8 cm above the dorsum and 3 to 9 cm proximal to the tip of the tail. Three latency trials were collected at each data point with the intensity of the radiant heat high enough to produce baseline latencies between 2.0 and 3.5 s. A cut-off latency of 12 s was employed to minimize tissue damage. The first four groups of rats were evaluated for full rppOFQ/N154–181 dose-response curves in the four following brain sites: vlPAG (0.725–5.8 μM), amygdala (0.014–11.6 μM), LC (0.725–5.8 μM), and RVM (0.725–5.8 μM) and were compared with vehicle treatment at 5, 15, and 30 min after microinjection of rppOFQ/N154–181. The full-length peptide (rppOFQ/N154–181) was dissolved in normal 0.9% saline and infused over 30 s in a 1-μl volume through an internal cannula (33 gauge; Plastics One). The second four groups of rats were evaluated for their sensitivity to systemic pretreatment of naloxone (5 mg/kg; 15 min prior to administration of the full-length peptide) in each site.

**Assay for Hyperalgesia.** In addition, hyperalgesia was assessed in the same paradigm as cited above by lowering the intensity of the radiant heat, which led to longer baseline latencies (8.81 ± 0.23 s). Consequently, the cut-off latency was increased to 20 s. Thus, this paradigm was capable of assessing both antinociception and hyperalgesia (pronociception). After the administration of rppOFQ/N154–181 at a dose of 5.8 μM, we examined all of the above-mentioned brain areas for hyperalgesic responses in separate groups of rats according to the same time course at which this dose yielded significant antinociceptive scores in these four sites.

**Antinociceptive Assay of Peptide Fragments**

**C-Terminal Fragment (rppOFQ/N174–181).** To determine which part of the full-length peptide is exerting antinociceptive effects, a separate group of rats were challenged in the PAG with an equimolar dose of either the full-length peptide (rppOFQ/N154–181: 11.7 μM) or a shortened C-terminal fragment (rppOFQ/N174–181: 11.34 μM) and examined in the same time course as above.

**N-Terminal Fragment (rppOFQ/N154–170 (OFQ2)).** To further determine the active part of the full-length peptide, we examined the N-terminal part of the peptide, (rppOFQ/N154–170). However, in light of the solubility factors of OFQ2, all stainless steel cannulae and tubing were presoaked in 20% albumin prior to implanting and testing. In all testing conditions OFQ2 was dissolved in 0.1% DMSO and immediately used for behavioral testing. Three brain areas (the lateral ventricle, the PAG, and the LC) were examined. All testing was performed in the same time course as above.

**Radioimmunoassay**

The full-length peptide (rppOFQ/N154–181) was synthesized and conjugated by Covance (Denver, PA) for rabbit immunization. The
antiserum (VO61) used was specific for the mid-portion of rppOFQ/N154–181, does not cross-react with rppOFQ/N154–170 (OFQ2), and showed minimal cross-reactivity with rppOFQ/N154–181. This suggests that the antiserum requires the intact RRR triple basic site for full reactivity. The antiserum also does not cross-react with the β-endorphin, dynorphin A, or OFQ/N. rppOFQ/N154–181 was iodinated using the hypochlorite method (Quigley et al., 1998). All synthetic peptides were obtained from Phoenix Pharmaceuticals (Belmont, CA).

**Tissue Isolation and Sample Preparation**

Individual brain tissues (two to three tissue blocks of the PAG, amygdala, and RVM) were extracted in 0.5 ml of 10% acetic acid, containing 0.5 mg/ml bovine serum albumin and protease inhibitors (1 μl/ml phenylmethyl sulfonylfluoride; Sigma-Aldrich). Samples were homogenized manually, frozen, and thawed three times. The tissue extracts were then centrifuged, and the resulting supernatants were dried under vacuum for peptide analyses. Samples were resuspended in 1 ml of 0.1% trifluoroacetic acid, pooled, and centrifuged, and a portion was injected onto a Vydac (214TP54) RP-HPLC column (C4, 300-Å pore size; The Separations Group, Hesperia, CA). The extracts were fractionated using an RP-HPLC system (Waters, Milford, NJ) with linear gradients of acetonitrile in 0.1% trifluoroacetic acid and a flow rate of 1 ml/min as described in the legend of Fig. 2. Fractions were vacuum dried prior to radioimmunoassay. Authentic rppOFQ/N154–181 was also fractionated by RP-HPLC, and the elution time is indicated (for further details, see Quigley et al., 1998).

**Statistics**

Appropriate t-tests and analyses of variance were performed. In addition, repeated measures analyses were performed for each paradigm for each site that analyzed dose/condition effects as one variable, and time course as the second variable. Tukey-corrected comparisons (p < 0.05) detected individual significant effects.

**Results**

The rat OFQ/N precursor could potentially encode several biologically active peptides, all of which are schematically diagramed in Fig. 1. To determine whether the full-length peptide, rppOFQ/N154–181 was produced in the brain, we fractionated extracts of PAG, RVM, and amygdala tissues by RP-HPLC and assayed the fractions for rppOFQ/N154–181 immunoreactivity. Figure 2 shows that the PAG, the RVM, and the amygdala contain rppOFQ/N154–181 immunoreactive material, with the major peaks eluting at 62 to 63 min and 66 to 67 min. Synthetic rppOFQ/N154–181 elutes at 62 to 63 min. Interestingly, the relative amounts of each immunoreactive peak differed among the brain areas. The RVM contained less total immunoreactivity and most of the immunoreactivity eluted later than authentic rppOFQ/N. The PAG and amygdala extracts contained similar amounts of both peaks. rppOFQ/N154–181 immunoreactivity was undetectable in the LC.

**rppOFQ/N154–181 Antinociception.** Significant differences in high intensity-radiant heat latencies were observed in viPAG (Fig. 3a), amygdala (Fig. 3b), the LC (Fig. 3c), and the RVM (Fig. 3d). In the viPAG and the amygdala, rppOFQ/N154–181 significantly elevated latencies in a dose-dependent manner with peak effects at 5 min and a duration of action of approximately 30 min (Fig. 4, a and b, respectively). Additionally, ceiling effects were observed in the amygdala. Although the responses were quite robust in the viPAG and amygdala, the viPAG failed to display antinociceptive effects at rppOFQ/N154–181 doses below 1.45 μM (Fig. 3, a and b). Additionally, it appeared that these antinociceptive effects were not due to any observable motor deficits in animals with cannulae directed at the amygdala or any other site.

In the LC and the RVM, rppOFQ/N154–181 produced dose-dependent responses (Fig. 3, c and d) that elicited a peak effect at 5 to 15 min and were completely gone in 30 min (Fig. 4, c and d, respectively). Longer postinjection times of 60, 90, and 120 min were examined, but failed to show behavioral effects in any site examined (data not shown). These data indicate multiple sites of supraspinal antinociceptive action in all four brain areas (Table 1). The peak effects of the two caudal sites (LC and RVM) were essentially equivalent at 5 and 15 min. Several missed LC cannulation sites (the parabrachial region, n = 3; reticular tegmental nucleus, n = 1; and trigeminal nerve tract of the mesencephalon, n = 1), failed to show antinociceptive effects (data not shown).

**Naloxone Sensitivity.** The full-length peptide (rppOFQ/N154–181) is capable of eliciting antinociceptive responses on the tailflick test at high thermal intensities yielding short baseline latencies with the antinociceptive magnitude in all four sites that appears to peak at approximately 6 to 8 s. This ceiling effect in the amygdala contrasts with greater magnitudes observed in these brain sites following other opioid peptides. We assessed whether systemic naloxone (5 mg/kg, s.c.) could block the antinociceptive actions of an effective dose (5.8 μM) of rppOFQ/N154–181 (Fig. 4). Site-specific dissociations were observed (Table 1). Significant blockade by
naloxone was observed in the vPAG (Fig. 4a) and amygdala (Fig. 4b) placements, with naloxone completely eliminating rppOFQ/N\textsubscript{154-181}-induced antinociception in these two sites. Although significant differences were observed in LC placements (Fig. 4c), naloxone failed to alter rppOFQ/N\textsubscript{154-181} antinociception after 5 and 15 min, and actually enhanced this LC response at 30 min. Similarly, although significant differences were observed in RVM placements across conditions and times, naloxone failed to alter rppOFQ/N\textsubscript{154-181} antinociception in the RVM (Fig. 4d). These results support

Fig. 3. Dose-response curves of the full-length peptide (rppOFQ/N\textsubscript{154-181}). Sprague-Dawley rats were cannulated in four different brain areas and tested on the high intensity-radiant heat tailflick assay, which yielded low baseline latencies (2.5 s). Dose-response curves are shown for the periaqueductal gray (0.725–5.8 \textmu M) (a); the amygdala (0.014–11.6 \textmu M) (b); the locus coeruleus (0.725–5.8 \textmu M) (c); and the rostro-ventral medulla (0.725–5.8 \textmu M) (d). Peak effects at 5 min are shown.

Fig. 4. Naloxone reversibility of the full-length peptide (rppOFQ/N\textsubscript{154-181}). The ability of the opiate antagonist naloxone to block rppOFQ/N\textsubscript{154-181}-induced analgesia is site-specific. Naloxone (5 mg/kg, s.c.) was administered 15 min prior to intracerebral administration of rppOFQ/N\textsubscript{154-181} (5.8 \textmu M; \textit{n} = 6/group). Normal analgesic baseline scores averaged between 2 and 3 s on the high intensity-radiant heat tailflick assay. a, naloxone reversibility in the PAG (\textit{p} < 0.001); b, similar naloxone reversibility in the amygdala (\textit{p} < 0.001). This high dose of naloxone failed to alter rppOFQ/N\textsubscript{154-181}-induced analgesia in the LC (c) or the RVM (d).
the notion that antinociceptive actions of rppOFQ/N\textsubscript{154–181} are mediated through classic opioid synapses in the amygdala and vIPAG, but not the LC or RVM.

**Hyperalgesia.** An opposite pattern of site-specific effects of rppOFQ/N\textsubscript{154–181} was observed in the hyperalgesia assay (Fig. 5). Using the low radiant heat tailflick assay, which produces longer baseline latencies, ppOFQ/N\textsubscript{174–181} in the vIPAG and amygdala produced significant antinociception on this test, no significant hyperalgesic responses were observed in the vIPAG or the amygdala (Fig. 5, a and b; Table 1). Thus, those sites (vIPAG and amygdala) that displayed naloxone-sensitive rppOFQ/N\textsubscript{154–181} antinociception failed to exhibit rppOFQ/N\textsubscript{154–181} hyperalgesia. In contrast, significant hyperalgesic effects in the LC (Fig. 5c) and the RVM (Fig. 5d) were observed on this measure at 15 and 30 min after injection. Thus, the LC and RVM display naloxone-insensitive rppOFQ/N\textsubscript{154–181} antinociception at a high radiant heat intensity and exhibited rppOFQ/N\textsubscript{154–181} hyperalgesia at low radiant intensities. Moreover, hyperalgesia was not seen with higher stimulus intensities due to a floor effect of ppOFQ/N\textsubscript{154–181}. These results indicate a double dissociation among these supraspinal sites in terms of opioid sensitivity to antinociceptive actions and the ability to elicit hyperalgesic responses after rppOFQ/N\textsubscript{154–181} administration.

**Characterization of rppOFQ/N\textsubscript{154–181} Fragments.** To determine which region(s) of rppOFQ/N\textsubscript{154–181} were physiologically active, we compared the full-length peptide (rppOFQ/N\textsubscript{154–181}) to the C-terminal 8 amino acids (rppOFQ/N\textsubscript{174–181}) (Fig. 6) in a part of the brain in which the parent peptide produced antinociception, the PAG. The C-terminal peptide (rppOFQ/N\textsubscript{174–181}) had no effect (Fig. 6). To further characterize this peptide, rats were then cannulated in the PAG, the LC, and the lateral ventricle and were challenged with the full-length peptide (2.5–5.8 μM) or the N-terminal fragment (OFQ2) (1.2–9.6 μM). In a recent study in mice (Mathis et al., 2001), it was shown that OFQ2 was more potent than rppOFQ/N\textsubscript{154–181} in the third ventricle. However, this is not the case in the rat. Antinociceptive effects in the PAG and LC in the rat show that OFQ2 produced significant antinociception at 5 min in the PAG and LC, yet its magnitude was less than rppOFQ/N\textsubscript{154–181} (Fig. 7, a and b). Using the ventricular route in the rat, the N-terminal fragment produced significant antinociception after 5 min (Fig. 7c) that persisted for 30 min. However this antinociceptive effect was not significantly greater than the antinociceptive effects of the full-length peptide, as was the case in the mouse (Mathis et al., 2001). Hence, these data indicate clear dose-dependent antinociception in all three sites, but also suggest species-dependent antinociceptive actions.

OFQ2 (rppOFQ\textsubscript{154–170}) antinociception elicited from PAG,

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**Table 1**

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<th>Location</th>
<th>Analgesia</th>
<th>Hyperalgesia</th>
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<td>Ventricle (i.c.v.)</td>
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<td>Yes</td>
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<td>Amygdala</td>
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<tr>
<td>RVM</td>
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**Fig. 5.** Hyperalgesia: low intensity tailflick assay. A separate group of rats were baselined at a lower intensity of radiant heat, which yielded higher tailflick latencies (8.81 ± 0.23). Analysis of variance revealed average baseline values that were significantly (p < 0.001) decreased in the LC (6.26 ± 0.2) at 15 min and significantly decreased in the RVM (5.97 ± 0.22 and 7.04 ± 0.4) at 15 and 30 min, respectively. These hyperalgesic effects were not seen in the two rostral sites, the PAG (a) or the amygdala (b). All four sites received the full-length peptide (rppOFQ/N\textsubscript{154–181}) at a dose of 5.8 μM (n = 6/group). Note: the same rats were not used in both the hyperalgesic assay and the analgesic assay.
LC, and icv placements was examined for naloxone reversibility and compared with rppOFQ/N154–181. Naloxone (5 mg/kg, s.c.) significantly blocked the peak effects (5 min) of OFQ2 and rppOFQ/N154–181 in the PAG (Fig. 8a), but only blocked the effects of OFQ2 when administered into the ventricle (Fig. 8c). Naloxone failed to affect OFQ2 or rppOFQ/N154–181-induced antinociception in the LC (Fig. 8b). These results suggest that the N terminus possesses some bioactivity, but it is not as potent as the full-length peptide.

**Discussion**

The PAG and amygdala yield opioid-sensitive rppOFQ/N154–181 antinociception, yet fail to exhibit a hyperalgesic response; whereas the LC and RVM yield opioid-insensitive rppOFQ/N154–181 antinociception and a hyperalgesic response. Therefore, these data indicate site specificity mediating the mechanism of antinociceptive actions and hyperalgesia. The presence of this double-dissociation serves as an important control in analyzing the specificity of the responses. These pairs of sites behave quite similarly in eliciting traditional opioid antinociception with greatest sensitivity to /H9262-selective ligands (Fang et al., 1986; Bodnar et al., 1988; Smith et al., 1988; Helmstetter et al., 1995; Pavlovic et al., 1996) as well as exhibit antinociceptive opioid synergy using morphine and /H9252-endorphin (Rossi et al., 1993, 1994; Pavlovic and Bodnar, 1998).

Interestingly, in the four rat brain sites examined, rppOFQ/N154–181 produced potent antinociception. However, the antinociceptive response in two of the cannulated sites (amygdala and PAG) was naloxone-reversible. Moreover, those sites that did not produce an opiate-reversible analgesia (the LC and the RVM) showed significant hyperalgesia. Is it possible for the same brain region to produce two different effects, both antinociception and pronociception? Previous research shows that pronociceptive and antinociceptive effects of OFQ/N are dissociable. The first line of evidence comes from earlier studies in our laboratory showing that antisense that targets the coding region of exon 1 of the ORL1 receptor clone decreases OFQ/N hyperalgesia when given icv, however, antisense targeting exons 2 and 3 of the same clone are ineffective icv. In contrast, ORL1 antisense to exons 2 and 3 decrease OFQ/N icv analgesia, whereas ORL1 exon 1 is ineffective in the RVM and LC (Rossi et al., 1997). In addition, other laboratories have confirmed the possibility of dissociation in a single brain region. In 1991, the Fields laboratory showed an interesting line of evidence that supports this dissociation in the RVM that is highly compelling (Fields et al., 1991). Fields and his coworkers demonstrated ON-OFF cells in the RVM that were both pain-facilitory and -inhibitory systems. This neurophysiological evidence for the role of pronociceptive and antinociceptive dissociation is re-
the LC, the PAG and ventricle showed naloxone reversibility for OFQ2. Our data now implicate RVM ppOFQ/N154

...markedly seen in further reports from Dr. Heinricher’s laboratory (Heinricher and Roychowdhury, 1997; Heinricher et al., 1999). Our data now implicate RVM ppOFQ/N154/H9262/N11005/H9262 administered 15 min prior to OFQ2 (9.61–170) showed naloxone reversibility (middle panel). Unlike the LC, the PAG and ventricle showed naloxone reversibility for OFQ2 (top panel). Additionally, naloxone significantly reversed the effects of rppOFQ/N154–170 in the PAG (top panel).

The ability of naloxone to reverse the antinociceptive actions of the full-length peptide raises interesting questions. We have shown that both the full-length peptide (rppOFQ/N154–181) and the N-terminal fragment (rppOFQ/N154–170) possess bioactivity, with the parent peptide showing more potency in most physiological tests. In contrast, the small C-terminal peptide shows no bioactivity. Nevertheless, the minimum peptide sequence for complete bioactivity is not known at present, but nonetheless active in the brain.

The biochemical isolation of rppOFQ/N154–181, combined with the demonstration of its complex physiological activities, provides evidence that biologically active peptides are processed from the precursor peptide, ppOFQ/N. Studies by Danielson et al. (2001) describe the cloning of OFQ and provide further evidence that ppOFQ/N evolved from a prodynorphin-like molecule and that the proOFQ/N molecule has no homologous C-terminal peptide, indicating that the mouse, rat, and human evolved separate, distinct peptides that are identical at the amino acid level. These studies from the Danielson and Dores laboratory, in addition to ours, provide insight into the underlying mechanisms by which novel and often divergent physiological functions arise in opioid and nonopioid systems (Danielson et al., 2001). Therefore, it is possible and probable, that the OFQ/N system may have evolved as a separate and distinct system for pain perception.

References


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