Peripheral and central actions of orphanin FQ (nociceptin) on murine colon

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Osinski, M. A., P. Bass, and E. A. Gaumnitz. Peripheral and central actions of orphanin FQ (nociceptin) on murine colon. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G125–G131, 1999.—Orphanin FQ (OFQ), also known as nociceptin, is a recently isolated endogenous peptide with a structure similar to the endogenous opioid peptides. The present study examines the actions of centrally administered OFQ on in vivo murine gastrointestinal and colonic transit as well as the actions of OFQ on the isolated colon. Intracerebroventricular injections of OFQ dose dependently inhibited colonic propulsive activity. OFQ inhibition of colonic propulsion was unaffected by coadministration of the competitive opioid receptor antagonist naltrindone. A subadditive response was observed when approximately equipotent doses of either morphine sulfate or the δ-agonist DPDPE were coadministered with OFQ. No subadditivity was observed with coadministration of the μ-agonist DAMGO, suggesting a functional interaction between OFQ and δ-opioid central pathways regulating colonic transit. High, but not low, doses of OFQ also inhibited the transit of a nonabsorbable charcoal marker through the stomach and/or small intestine. OFQ potently contracted isolated colon preparations; contractile activity was abolished by TTX or chlorpromazine. Our results suggest that OFQ may be an important peptide ligand acting on a novel inhibitory neural pathway that modulates gastrointestinal transit.

BOTANICAL ALKALOIDS SUCH AS morphine, as well as synthetic opioid compounds, have severe constipating and antidiarrheal activity in humans and animals. These gastrointestinal effects can limit the use of these compounds as analgesics. The constipating effects of opiate alkaloids result from actions on smooth muscle activity (both tone and contractility) as well as inhibition of mucosal fluid secretion (reviewed in Ref. 6). Motility changes by opioids can be either stimulatory or inhibitory, depending on the species and preparation utilized; however, the net effect of opioids is to prolong transit of luminal contents through the gastrointestinal tract. Thus adverse gastrointestinal side effects of opioid compounds limit their full potential as analgesics.

Three types of opioid receptors (μ, κ, and δ) have been defined based on the results of radioligand binding assays (16, 26), functional experiments (18), and, finally, the molecular cloning of the receptor cDNA sequences (3, 8, 13, 35). Opioid receptors are members of the G protein-coupled receptor superfamily, and their activation results in an inhibition of adenylyl cyclase and a decrease in intracellular cAMP levels. The elucidation of the opioid receptor cDNA sequences allowed investigators to search nucleic acid libraries for other opioid receptor types or subtypes, the existence of which had been suggested from pharmacological studies. Recently, a novel member of the opioid receptor family was discovered using low-stringency hybridization techniques (10) and/or RT-PCR with degenerate primers to conserved regions of the three opioid receptor cDNAs (22). Shortly thereafter, this receptor’s endogenous peptide ligand, termed orphanin FQ (OFQ) (31), or, alternatively, nociceptin (19), was simultaneously isolated. The cloned human OFQ receptor bears approximately the same percent amino acid identities to the human μ-, δ-, and κ-opioid receptors as these three receptors do to each other. Like the opioid receptors, stimulation of the OFQ receptor results in suppression of adenylyl cyclase activity. Interestingly, the OFQ receptor does not significantly bind any of the endogenous opioid peptides, and naloxone, a competitive opioid receptor antagonist, does not antagonize the binding of OFQ to its receptor. OFQ and its receptor are expressed widely throughout the rat central nervous system (1, 2, 10, 14, 22). The expression patterns of the OFQ receptor as well as the results of pharmacological investigations suggest that OFQ might mediate a variety of physiological processes, including hyperalgesia (19, 31), locomotion (7), and spatial learning (33). Interestingly, mice lacking the OFQ receptor do not display significant changes in either nociceptive threshold or locomotor activity but do display impairment in hearing ability (24).

The endogenous peptide ligand for the OFQ receptor is a 17-amino-acid peptide recently isolated from porcine hypothalamus and also rat brain. The sequence of OFQ is most similar to the endogenous opioid peptide dynorphin A, a κ-opioid receptor agonist. The amino-terminal sequence of OFQ, Phe-Gly-Gly-Phe, differs from the canonical Tyr-Gly-Gly-Phe tetrapeptide sequence, which begins all other mammalian opioid peptides. Also, the carboxy-terminal portion of OFQ contains several basic residues, a property shared by dynorphin A and β-endorphin. In contrast to the other opioid peptides, truncation of the carboxy-terminal end of OFQ results in loss of biological activity and lowered affinity of OFQ for its receptor (30). These results might explain the selectivity of OFQ for its receptor and not...
METHODS

Animals. All procedures utilizing animals were approved by the Animal Care Committee of the University of Wisconsin-Madison. Male ICR mice (25–34 g) were obtained from Harlan Sprague Dawley (Madison, WI) and were housed in groups of three to five in plastic cages. Mice were maintained on a 12:12-h light-dark cycle with lights on beginning at 6:00 AM. Food and water were provided ad libitum up until the time of testing, except for those animals in the gastrointestinal transit study. Those animals were fasted for 24 h before testing.

Compounds and administration. All substances used in this study were purchased from either Research Biochemicals (Natick, MA) or Sigma Chemical (St. Louis, MO). Intracerebroventricular injections were performed as described by Laursen and Belknap (15) on mice lightly anesthetized with ether. Light anesthesia was defined as loss of the animal’s righting reflex. Animals generally recovered from anesthesia within 3 min. All compounds were dissolved in sterile saline and administered intracerebroventricularly 5 min before the insertion of the glass rod, one end of which was fire-polished so as to be rendered atraumatic. After bead insertion, mice were placed in individual plastic cages lined with white paper to aid visualization of bead expulsion. The time required for expulsion of the glass bead was determined noncumulatively, since preliminary experiments revealed tissue desensitization to OFQ when administered with a cumulative dosing protocol. Time between OFQ doses was 15 min. In experiments testing for antagonism of the contractile response of OFQ, all antagonists were equilibrated with tissues for at least 20 min before testing for interaction with the OFQ receptor.

Data analysis. Data are expressed as means ± SE or with 95% confidence intervals, as indicated in the text. In vivo data were analyzed with one-way ANOVA followed by Dunnett’s multiple comparison procedure to test for significant differences among means. Concentration-response curves from in vitro experiments were analyzed with nonlinear regression techniques. P < 0.05 were chosen as evidence of statistical significance. Computer software packages utilized were Minitab for Windows, release 11.13 (Minitab, State College, PA), and Prism (GraphPad Software, San Diego, CA).

RESULTS

Centrally administered OFQ inhibits colonic transit. Intracerebroventricular injection of OFQ resulted in dose-dependent increases in bead expulsion times (Fig. 1). There was no difference in bead latency times between animals that were administered an intracerebroventricular injection of saline and animals that did not receive intracerebroventricular injections (10.8 ± 1.0 vs. 8.8 ± 1.5 min, respectively; P > 0.05), indicating that intracerebroventricular injection of saline alone had no significant effect on bead expulsion times. Coadministration of 2.5 nmol of naltrexone, a competitive opioid receptor antagonist, and 5 nmol OFQ had no significant effect on the action of 5 nmol OFQ alone (36.4 ± 3.8 min with OFQ + naltrexone vs. 39.2 ± 4.9 min with OFQ alone; P > 0.05). This dose of naltrexone was sufficient to antagonize the colonic transit changes caused by an approximately equipotent dose of morphine sulfate (Fig. 2A). Furthermore, naltrexone by itself had no effect on bead expulsion time (8.2 ± 1.2 min with naltrexone vs. 10.0 ± 1.0 min with saline; Fig. 2A).

Subadditive response to coadministered morphine and OFQ. Because it has been reported that OFQ may function as an endogenous antiopioid peptide when examined in assays of cutaneous nociception (20, 21), we sought to determine if this mechanism also exists in...
the central control of colonic transit. Coadministration of 5 nmol OFQ and 0.15 nmol morphine sulfate resulted in bead latency that was less than predicted (Fig. 2A).

Subadditive responses observed with coadministered OFQ and δ- but not µ-opioid receptor agonists. Although morphine is often regarded as a µ-receptor-preferring agonist, it also has activity at δ- and κ-opioid receptors. Because of the potential nonselective activity of morphine at opioid receptors, we wanted to determine if the subadditive response we observed with morphine and OFQ might be due to morphine’s µ- and/or δ-receptor activity. Therefore, we conducted similar coadministration experiments with more selective opioid agonists. Coadministration of the µ-selective agonist [d-Ala²,N-Me-Phe⁴,Gly-ol⁵]enkephalin (DAMGO) with 5 nmol OFQ resulted in a bead latency that approximated the latency expected from an additive effect of the two compounds given individually (Fig. 2B). Coadministration of the δ-selective opioid agonist [d-Pen²,d-Pen⁵]enkephalin (DPDPE) with OFQ, however, resulted in a bead latency suggestive of a subadditive effect (Fig. 2C). Thus the subadditive effect observed in response to morphine and OFQ coadministration may reflect a possible δ-receptor agonistic

Fig. 2. Subadditive responses occur to coadministered OFQ and morphine sulfate as well as to coadministered OFQ and [d-Pen²,d-Pen⁵]enkephalin (DPDPE) but not to coadministered OFQ and [d-Ala²,N-Me-Phe⁴,Gly-ol⁵]enkephalin (DAMGO). In A–C, each pair of horizontal solid lines represents the upper and lower 95% confidence limits for the mean bead latency of control (vehicle-injected) animals (7.7–12.3 min). All bars represent mean responses (+ SE) obtained from 8 to 14 animals per group. A: coadministered morphine sulfate (MS; 0.15 nmol) and OFQ (5 nmol) resulted in a subadditive response (MS + OFQ). Extrapolated bar was created by adding the mean values of the “MS 0.15” and “OFQ 5.0” groups. Likewise, the “error estimate” for the extrapolated bar was obtained by adding the error estimates from these 2 groups. NTX 2.5, bead latency in response to 2.5 nmol naltrexone; MS 0.15, bead latency in response to 0.15 nmol morphine sulfate; MS + NTX, bead latency in response to coadministration of 0.15 nmol morphine sulfate and 2.5 nmol naltrexone; OFQ 5.0, bead latency in response to 5 nmol OFQ. B: coadministration of DAMGO (0.002 nmol) and OFQ (5 nmol) resulted in a response (DAMGO + OFQ) similar to the expected response (extrapolated, calculated as described in A). DAMGO 0.002, bead latency in response to 0.002 nmol DAMGO; OFQ 5.0, bead latency in response to 5 nmol OFQ. C: coadministered DPDPE (1.25 nmol) and OFQ (5 nmol) resulted in a response (DPDPE + OFQ) less than predicted (extrapolated, calculated as described in A). DPDPE 1.25, bead latency in response to 1.25 nmol DPDPE; OFQ 5.0, bead latency in response to 5 nmol OFQ. * Statistically different from control group at P < 0.05.
property of morphine. Thus, in contrast to the nonselective antiopioid effects of OFQ in nociception, subadditive colonic responses to coadministered OFQ and opioid compounds only occurred with the δ-receptor agonist DPDPE.

Centrally administered OFQ delays stomach emptying/intestinal transit. Low doses (1.25 and 2.5 nmol) of OFQ did not affect the progress of the charcoal front in the small intestine (Fig. 3). Five and ten nanomole doses of OFQ, however, significantly decreased gastrointestinal transit (25.1 ± 5.2%, P < 0.01, and 30.8 ± 5.2%, P < 0.05, respectively) compared with the control (saline-injected) group (52.9 ± 4.4%). Because the charcoal suspension was introduced by gavage into the stomach, and not by a duodenal catheter, differential activity of OFQ on stomach emptying and/or intestinal transit could not be elucidated.

OFQ contracts isolated murine colon. OFQ concentration dependently contracted isolated segments of proximal and distal colon suspended in the longitudinal axis (Fig. 4). No significant differences in either the potency or the efficacy of OFQ were observed between proximal and distal segments of colon (Fig. 5). Maximal OFQ-evoked contractile responses of the proximal and distal segments were 43.1% (95% confidence intervals, 39.6–46.5%) and 42.2% (39.6–44.7%) of the maximal tissue response elicited with 30 µM carbachol. EC50 values of OFQ were 0.48 nM (0.28–0.83 nM) and 1.02 nM (0.71–1.45 nM) in the proximal and distal colonic segments, respectively.

The ability of a variety of antagonists to affect contractions elicited by 10 nM OFQ was examined in colonic segments. TTX (1 µM) completely abolished OFQ activity (Fig. 4), indicating that OFQ acts through enteric nerves and not directly on smooth muscle. Chlorpromazine (30 µM) also abolished OFQ-induced contractions as well as contractions elicited by carbachol, presumably due to chlorpromazine’s actions on intracellular calcium regulatory mechanisms. None of the following compounds had any significant effects on OFQ-elicited contractions (data not shown): naltrexone (1 µM), atropine (1 µM), indomethacin (2 µM), picro-
toxin (10 µM), bicuculline (30 µM), strychnine (10 µM), sulpiride (10 µM), and SCH-23390 (10 µM). Therefore, neurogenic OFQ-mediated contraction of isolated colon occurs independently of the opioidergic, muscarinic, prostanoid, GABAergic, glycnergic, and dopaminergic neurotransmission pathways.

**DISCUSSION**

The results of this study show that 1) OFQ administered into the cerebral ventricles of mice inhibits colonic transit in a naltrexone-insensitive manner, 2) subadditivity of in vivo colonic responses, potentially indicative of functional antagonism between opioids and OFQ, exists between central opioid and OFQ receptor-mediated control of colonic transit, 3) centrally administered OFQ decreases gastric emptying and/or small intestinal transit and, 4) OFQ contracts isolated mouse colon through a neurogenic, nonopioid-mediated mechanism.

The actions of OFQ have been studied at the molecular, cellular, and whole animal level. The molecular (i.e., receptor) level activity of OFQ is distinct from the activity of other opioid receptor ligands such as morphine and related opioid alkaloids, the synthetic opioids, and the endogenous opioid peptides. These opioid compounds bind poorly or not at all to membranes from COS-7 cells transfected with the OFQ receptor (14). In contrast to the differences between OFQ and other opioid compounds at the receptor level, some of the actions of OFQ at the cellular level are similar to those of the other opioids. Activation of the OFQ receptor transfected in Chinese hamster ovary cells inhibits forskolin-stimulated cAMP production (19, 31). Also, OFQ stimulation of its receptor has been shown to activate inwardly rectifying potassium channels in several regions of the brain (4, 36). In addition, modulation of N-type calcium channels by OFQ has been shown (5).

Given the disparities between the actions of OFQ and other opioids at the molecular and cellular levels, it is not surprising that the actions of OFQ and other opioids also differ in their functional activity. Paradoxically, OFQ has been reported to possess both analgesic and hyperalgesic properties. For example, in the mouse tail-flick assay of nociception, intracerebroventricular injection of OFQ was originally reported to have hyperalgesic activity (31). However, Rossi and co-workers (32) have shown that OFQ can display either naloxone-sensitive analgesic activity or biphasic hyperalgesic/analgiesic activity, depending on the intensity of the stimulus. Yet another report suggests that OFQ has no inherent analgesic activity but possesses the ability to reverse µ-, κ-, and δ-opioid receptor-mediated analgesia (21). Therefore, although the similarities of the receptor sequences and some of the cellular functions of OFQ and other opioids might suggest similar functions a priori, the data reported so far suggest otherwise. The results of the present study show that actions of OFQ on the murine gastrointestinal tract are qualitatively similar to the previously reported gut actions of opioids such as morphine and the endogenous opioid peptides (28, 29), with the important exception being the naltrexone insensitivity of effects of OFQs.

In contrast to the many reports of OFQ action in the central nervous system, few studies have investigated the role of OFQ in gastrointestinal physiology. The message for the OFQ receptor has been shown to be widely expressed throughout the porcine gastrointestinal tract (25). Zhang and co-workers (40) have demonstrated that OFQ inhibits electrically evoked twitches of longitudinal muscle from the guinea pig ileum, a classic bioassay for opioid activity. The inhibition was naloxone insensitive. OFQ has also been shown to contract isolated strips of rat colon oriented in either the circular or longitudinal axis, with OFQ evoking greater contractions in the proximal colon (39). Preliminary reports of OFQ's actions on in vivo gastrointestinal function suggest that OFQ is stimulatory to gastrointestinal smooth muscle. OFQ given intravenously to anesthetized rats has been shown to increase intragastric pressure (11). Similarly, OFQ given intravenously to rats (state of consciousness unknown) caused colonic contractions (34). All of the aforementioned studies reveal that OFQ has actions similar to those of endogenous and exogenous opioid compounds when tested in similar assays, with the important difference being that OFQ's actions are naloxone insensitive. Experimental animals other than the mouse have been extensively studied for the actions of opioids on gastrointestinal function. The activity of gastrointestinal smooth muscle can be described as changes in transit, tone, or phasic activity (38). In the present study, we interpret the results of the colonic bead assay as alterations in colonic transit. Similarly, the charcoal meal assay reflects changes in gastrointestinal transit that may result from drug effects on stomach emptying and/or small intestinal transit. In other species, delay of gastrointestinal transit may result from increased smooth muscle tone as well as increased phasic activity (27). Although quantitative differences in opioid effects on gut function have been observed, e.g., contraction of isolated smooth muscle strips vs. inhibition of intestinal transit, there is an overall agreement on the qualitative effect of opioids on the gut, namely, inhibition of transit. For the sake of brevity, we have mainly limited our discussion to previous reports of opioid effects on murine gut.

Using essentially the same assay of in vivo colonic propulsion employed in the present study, Raffa et al. (29) have shown that intracerebroventricular injection of morphine, DAMGO, and the δ-receptor agonist DPDPE all inhibit colonic propulsive activity in a dose-dependent, naloxone-reversible fashion. Our finding that OFQ also inhibited colonic transit in a naltrexone-insensitive manner was surprising, since OFQ has been shown to exert antiopioid effects in other tests, e.g., cutaneous nociception (20). The presence of a subadditive response to coadministered OFQ and µ-opioid agonist DPDPE but not to coadministered OFQ and µ-selective agonist DAMGO was also unexpected given the results of a previous study that demonstrated the presence of functional antagonism of antinociception
Our data suggest that OFQ might interact with a central δ-opioid pathway regulating colonic motility. The differences between the OFQ/δ-opioid subadditive response that we observed and the nonselective opioid/OFQ functional antagonism of nociception may reflect differences in the central pathways responsible for colonic antitranist and pain transmission or perhaps differences in animal strain responsiveness. Coadministration of a δ-selective antagonist, such as naltindole, with OFQ would provide additional evidence for the involvement of δ-opioid receptors in modulating the central gastrointestinal actions of OFQ. We did not test the effects of k-agonists or of δ-agonist/OFQ coadministration on colonic propulsion, so a potential role for morphine's k-opioid activity cannot be excluded. This seems unlikely given the poor efficacy of k-agonists on gastrointestinal motility in rats and mice when given supraspinally.

Alternatively, morphine and OFQ might be acting through separate neural pathways that converge onto a third pathway that in turn affects inhibition of colonic motility. The combined stimulation of this hypothetical component by morphine and OFQ could result in saturation or desensitization of its ability to inhibit colonic motility and the appearance of a subadditive response to coadministration of morphine and OFQ. The intestinal antisecretory effect of morphine has been shown to be indirect, involving multiple neurotransmitters and their receptors, suggesting that a parallel mechanism exists in the peripheral nervous system (6). Isobolographic analysis of the effects of multiple combinations of morphine and OFQ will be necessary to definitively investigate synergistic or subadditive effects of OFQ and opioids on both gastrointestinal as well as nociceptive function.

Centrally administered OFQ also delayed gastric emptying and/or small intestinal transit of the nonabsorbable charcoal suspension. It has been previously shown that intracerebroventricular injection of morphine or DADLE (an enkephalin analog with δ-opioid activity) delayed intestinal transit in mice (37). These results were confirmed and extended by Porreca and colleagues (28), who also showed that inhibition of transit was mediated by μ- and δ-opioid at the level of the spinal cord. The lack of dose dependency displayed by OFQ on gastrointestinal transit may reflect the need for higher doses of OFQ to achieve maximal inhibition of transit. This seems unlikely, since a trend to decreased inhibition of transit was observed at the 10 nmol dose of OFQ. Another explanation for the lack of dose dependency may be that OFQ is differentially modulating separate central pathways that control stomach emptying and intestinal propulsion.

The action of OFQ on in vitro murine colon is no less intriguing than its in vivo effects. Alkaloid opioids such as morphine as well as the endogenous opioid peptides potently contract in vitro distal colon preparations from rat (23) and mouse (9). The opioid-evoked contraction of mouse colon is abolished by TTX and naloxone, indicating that contraction occurs through opioid receptors on enteric neurons. Furthermore, the contractile responses of the murine colon to opioids are unaffected by muscarinic, adrenergic, histaminergic, or serotoninergic antagonists but potently inhibited by indomethacin (9), implying the involvement of a prostaglandin-mediated pathway. In our study, prostaglandins seem not to be involved in the contractile response evoked by OFQ, since 2 µM indomethacin did not affect the colon's response to OFQ. Our results in this study, as well as the results of others (39) obtained in the rat, suggest that OFQ acts through a novel neurogenic mechanism in the colon.

In summary, we have shown that the opioid-like peptide OFQ acts in a multifactorial, naloxone-insensitive manner in the murine gastrointestinal tract, inhibiting both gastrointestinal and colonic transit in vivo, while contracting isolated colon in vitro. Thus OFQ and its receptor may represent a novel inhibitory peptidergic pathway important in the regulation of gastrointestinal transit.

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