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Orphanin FQ/nociceptin and [Phe$^1\Psi$(CH$_2$-NH)Gly^2] nociceptin(1-13)-NH$_2$ stimulate gastric motor function in anaesthetized rats

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1 Orphanin FQ/nociceptin (OFQ/N) is a preferred endogenous ligand for the orphan opioid receptor-like-1 receptor. This peptide has been reported to increase intestinal, but not gastric, motor activity.

2 In the present study, OFQ/N (0.6–60 nmol kg^{-1} i.v.) increased intragastric pressure and antral contractility and, as expected, decreased blood pressure in anaesthetized rats.

3 The gastric motor effects of OFQ/N (6 nmol kg^{-1}) were not affected by inhibition of nitric oxide synthase or opioid receptor blockade.

4 OFQ/N (6 nmol kg^{-1}) evoked gastric motor increases and hypotension were not affected by prior administration of its derivative [Phe$^1\Psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ unless the pseudopepotide was administered shortly (5 min) prior to OFQ/N. This putative antagonist (6–300 nmol kg^{-1}) alone increased antral motility with approximately 100 fold lower potency than OFQ/N.

5 Neither bilateral vagotomy nor spinal cord transection altered OFQ/N-evoked increases in intragastric pressure and antral contractility.

6 In conclusion, OFQ/N induces gastric motor excitation in addition to its known effects to increase intestinal motility. The gastric responses to OFQ/N are not dependent on 'classical' opioid receptor activation or nitric oxide, similar to the case for the intestines. The primary site of action of OFQ/N on the stomach is probably *via* enteric nerves, since central descending vagal or sympathetic pathways are not necessary for OFQ/N to increase gastric motility. The gastric motor effects of the derivative [Phe$^1\Psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ are similar to OFQ/N, although with lower potency. The effects of the derivative as a partial agonist or antagonist in different experimental paradigms may reflect tissue OFQ/N receptor reserve.

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Abbreviations: [FG]OFQ/N(1-13-NH$_2$), [Phe$^1\Psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$; HR, heart rate; IGP, intragastric pressure; MAP, mean arterial pressure; MMI, minute motility index, L-NAME, N^G-nitro-L-arginine; OFQ/N, orphanin FQ/nociceptin

Introduction

Orphanin FQ/nociceptin (OFQ/N) is a preferred endogenous ligand for the orphan or opioid receptor-like-1 receptor (Bunzow *et al*., 1994; Mollereau *et al*., 1994). The OFQ/N receptor mRNA is expressed in the porcine (Osinski *et al*., 1999b) and rat (Wang *et al*., 1994) intestine and OFQ/N dose-dependently increases contractility in isolated colon strips (Osinski *et al*., 1999a; Yazdani *et al*., 1999). Since these effects are abolished by tetrodotoxin, veratridine and serosal application of benzalkonium chloride, it is concluded that the receptor is present on myenteric plexus nerves, rather than smooth muscle (Osinski *et al*., 1999a; Yazdani *et al*., 1999). Supportive of this, OFQ/N-immunoreactive fibres have been identified in the myenteric plexus of rat colon (Yazdani *et al*., 1999). Thus, the OFQ/N receptor apparently modulates neural control of colonic motility.

A tridecapeptide analogue of OFQ/N, [Phe$^1\Psi$(CH$_2$-NH)Gly^2]nociceptin-(1-13)-NH$_2$ (referred to as [FG]OFQ/N(1-13)-NH$_2$) is the first reported selective antagonist to prevent the binding of the endogenous ligand OFQ/N at the orphan opioid-like receptor. [FG]OFQ/N(1-13)-NH$_2$ has been characterized as a OFQ/N antagonist in guinea-pig ileum and mouse vas deferens preparations (Guerrini *et al*., 1998). However, the derivative behaved as a pure agonist to inhibit cyclic AMP in Chinese hamster ovary cells expressing OFQ/N receptor (Butour *et al*., 1998) and a partial agonist to activate inwardly rectifying potassium channels in rat periaqueductal gray neurons (Chiou, 1999). Evidence *in vivo* also suggests that [FG]OFQ/N(1-13)-NH$_2$ is an agonist. For example, central intracerebroventricular administration of the derivative results in similar cardiovascular responses as OFQ/N, that is, bradycardia, hypotension and water diuresis (Kapusta *et al*., 1999). It also mimics the antinociceptive effects of OFQ/N when given intrathecally (Xu *et al*., 1998). Recently, it was reported that [FG]OFQ/N(1-13)-NH$_2$ increased contractility of mouse and rat colonic muscle strips similar to OFQ/N (Menzies *et al*., 1999). Therefore, there is increasing evidence that [FG]OFQ/N(1-13)-NH$_2$ is a full or partial agonist at the OFQ/N receptor both *in vivo* and *in vitro* preparations.

To date, the majority of data on the motility effects of OFQ/N are based on colonic and ileal responses to the peptide. This is surprising since the receptor transcripts are found throughout the gastrointestinal tract (Osinski *et al*., 1999b).

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However, Yazdani *et al*. (1999) noted that OFQ/N had no effect on gastric motility *in vitro*. An agent that selectively altered intestinal motility would be viewed differently than one that promoted motility throughout the gastrointestinal tract. Therefore, we have assessed the effects of systemic administration of OFQ/N or [FG]OFQ/N(1-13)-NH_2 on gastric motor function.

Our results demonstrated a potent effect of OFQ/N to increase gastric contractility. Therefore, we attempted to partially characterize the mechanism for this effect by using receptor antagonists, [FG]OFQ/N(1-13)-NH_2 (putative OFQ/N antagonist), naloxone (an opioid receptor antagonist) and NG-nitro-L-arginine methyl ester (L-NAME; a nitric oxide synthase inhibitor).

Finally, since increased gastric contractility in response to OFQ/N is in direct conflict with a previous study in isolated gastric muscle strips (Yazdani *et al*., 1999), we hypothesized that OFQ/N may act on autonomic pathways regulating gastric motility. Several lines of evidence are compatible with this idea. For example, OFQ/N-immunoreactive neurons are present in prevertebral sympathetic ganglia, and its receptor mRNA is detected in both para- and prevertebral ganglia (Kummer & Fischer, 1997). In addition, OFQ/N increased colonic motility when given by intracerebroventricular administration (Osinski *et al*., 1999a), which suggests that it has effects on central outflow to the gut. Therefore, we compared gastric motor effects of systemic OFQ/N before and after bilateral vagotomy and spinal cord transection. A preliminary account of these studies has been presented in abstract form (Krowicki *et al*., 1998).

Methods

Animals

Male-Sprague-Dawley rats (220–430 g) purchased from Charles River Laboratories (Wilmington, MA, U.S.A.), were used in all experiments. Food was withheld 12 h before experiments but the animals had free access to tap water. All experiments were performed with the approval of the Louisiana State University Health Sciences Center Animal Care and Use Committee.

Instrumentation and surgery

The animals were initially anaesthetized with ketamine and xylazine mixture (i.m. 50 and 5 mg kg^{-1} respectively) and a separate indwelling cannulae were placed in the left femoral artery (for blood pressure recording) and vein for administration of alpha-chloralose (i.v., 80 mg kg^{-1}) 25 min later. Blood pressure was monitored *via* a left arterial cannula connected to a pressure transducer (Viggo-Spectramed, model P23XL, Oxnard, CA, U.S.A.) and polygraph (model 7E, Grass Instrument Co. Quincy, MA, U.S.A.). Heart rate (HR) was monitored by tachograph driven by the pressure wave (model 7P4H, Grass Instrument Co. Quincy, MA, U.S.A.). Urethane (i.v., 600 mg kg^{-1}) or xylazine (i.v., 2.5 mg kg^{-1}) were used to maintain full surgical anaesthesia in the presence of alpha-chloralose. The trachea was cannulated with PE-160 tubing and, if necessary, animals were artificially ventilated using a small animal respirator (Kent Scientific Corp., Litchfield, CT, U.S.A.). Intragastric pressure (IGP) was continuously recorded using a latex balloon placed in the stomach. The balloon was made from the smallest digit of a small sized latex glove (Safeskin, Boca Raton, FL, U.S.A.). A length of PE-160 tubing was inserted into the stomach through the fundus *via* a small opening and a purse-string suture secured the tubing in place. Additionally, a small strain gauge (termed a figure 7 arch, 120 mOhm, Warren Research Products, Charlestown, SC, U.S.A.) was mounted on the surface of the stomach for monitoring of smooth muscle contractile activity of the terminal antrum.

Bilateral vagotomy was performed in five animals at the midcervical level. Briefly, the vagi were carefully separated from the left and right common carotid arteries and silk snares were loosely placed around them, then vagotomy was achieved by avulsion. Transection of the cervical spinal cord was performed in four animals at the level of the medullospinal transition region. To prevent activation of nociceptive reflexes, 0.5 ml of 2% lidocaine HCl (Butler, Columbus, OH, U.S.A.) was injected with a 25-gauge needle directly into the exposed spinal cord in several locations, then 0.5 cm of the cord was excised to ensure complete interruption of spinal efferents. These acute, terminal procedures were performed in the presence of full surgical anaesthesia. Vagotomy or spinal cord transection was performed after typical gastric motor responses to the peptide were obtained, followed by repeat administration of OFQ/N 2 h later. The animals were allowed to stabilize for at least 45 min prior to further experimentation. The intragastric balloon was then inflated with warm saline to an imparting pressure of approximately 5 cmH_2O. Rectal temperature was maintained between 37.0 and 37.5°C with a radiant heat lamp.

Drugs

Orphanin FQ/Nociceptin (Phoenix Pharmaceuticals, Mountain View, CA, U.S.A. or Bachem California Inc., Torrance, CA, U.S.A.) and [FG]OFQ/N(1-13)-NH_2 (Phoenix Pharmaceuticals, Mountain View, CA, U.S.A.) were dissolved in 0.15 M NaCl with 0.2% radioassay grade bovine serum albumin (Sigma Chemical Co., St. Louis, MO, U.S.A.) to reduce loss of drug due to low affinity binding to syringe etc. L-NAME (10 ng kg^{-1}) and naloxone (10 mg kg^{-1}), both from Sigma Chemical Co., St. Louis, MO, U.S.A. were dissolved in 0.15 M NaCl. The doses selected for these commonly used agents are based on the literature and have been shown to be effective. The agents and control injections, which were identical in terms of the diluent minus the drug, were administered *via* femoral vein as bolus injections in a volume of 1 ml kg^{-1}.

Assessment of gastric motor and cardiovascular function

For peak response in IGP (maximum difference from baseline) and antral contractility, the changes from baseline were calculated. Minute motility index (MMI) was calculated for 2 min before and after injection (Ormsbee & Bess, 1976) as follows. $MMI = (N1 \times 1) + (N2 \times 2) + (N3 \times 4) = (N4 \times 8)$; where N1 is the number of contractions with tension from 1–2 g; N2 is the number from 2–4 g; N3 is the tension from 4–8 g and N4 is over 8 g of contractile force. Additionally, the area of the response in IGP for each treatment was calculated with a computerized imaging system (Imaging Research Inc., Ontario, Canada). Blood pressure is expressed as mean arterial pressure (MAP) and was calculated by adding 1/3 of the pulse pressure to the diastolic pressure.

Statistical analysis

Results are expressed as means ± s.e. Significance of differences was assessed by paired *t*-test or one-way analysis of variance

followed by Student-Newman-Keuls or Dunn's test. P values <0.05 were considered statistically significant.

Results

Gastric motor effects of OFQ/N and [FG]OFQ/N(1-13)-NH$_2$

The effects of vehicle and OFQ/N, injected randomly i.v. at doses from 0.06–60 nmol kg^{-1}, on gastric motor function are shown in Figures 1–3. Antral contractile activity significantly increased in response to OFQ/N at a dose of 0.6 nmol kg^{-1}, with the peak effect at 6 nmol kg^{-1} and no further increase in effect at 60 nmol kg^{-1} (Figure 1). Moderate, but significant, increases in peak (Figure 2) and total (Figure 3) IGP were noted at doses of OFQ/N from 0.6–60 nmol kg^{-1}. The values in Figures 1–3 are shown as changes from baseline, for which the baselines are as follows (peak IGP cmH$_2$O/antral MMI) – vehicle; (4.3±0.2/3.3±0.5); 0.06 nmol kg^{-1} (4.2±0.3/2.6±1.1); 0.6 nmol kg^{-1} (3.9±0.4/3.7±1.6); 6 nmol kg^{-1} (4.1±0.3/6.5±1.8); 60 nmol kg^{-1} (4.2±0.4/9.7±2.5).

Figure 4A shows a trace recording from a representative experiment in which OFQ/N was administered i.v. at a dose of 6 nmol kg^{-1}. Immediately after injection of the peptide, a marked increase in antral motility of +22 MMI occurred and returned to baseline within 4 min. A moderate increase in IGP (peak change: +3.5 cm H$_2$O; area of the response: 0.56 cm^2) occurred immediately after injection and returned to baseline within 3 min. Also shown in Figure 4 are the HR and MAP responses to OFQ/N. Overall, OFQ/N (0.6–60 nmol kg^{-1}) evoked significant dose-related decreases in HR and MAP (data not shown).

The effects of vehicle and [FG]OFQ/N(1-13)-NH$_2$, injected randomly i.v. at doses from 0.6–300 nmol, on gastric motor function are shown also in Figures 1–3. These data show that [FG]OFQ/N(1-13)-NH$_2$ evoked significant increases in antral contractility (Figure 1) at doses of 6–300 nmol kg^{-1}, with no significant differences between these doses. Small but significant increases in IGP (both the peak change and the total area of the response; Figures 2 and 3, respectively) occurred at doses of 6–300 nmol kg^{-1}. The values shown in Figures 1–3 are shown as changes from baseline for which the baselines are as follows (peak IGP cmH$_2$O/antral MMI): vehicle; (4.6±0.2/4.5±1.2); 0.6 nmol kg^{-1} (4.5±0.2/6.6±2.5); 6 nmol kg^{-1} (4.9±0.1/13.1±4.0); 42 nmol kg^{-1} (3.8±0.3/2.7±1.5) 300 nmol kg^{-1} (4.6±0.4/3.6±1.3).

Figure 4B shows a trace recording from a representative experiment in which [FG]OFQ/N(1-13)-NH$_2$ is administered i.v. at a single dose of 6 nmol kg^{-1}. Immediately after administration of the derivative, an increase in antral motility (+22 MMI) was observed, which returned to baseline within 3 min. A slight increase IGP (peak change was +1.0 cm H$_2$O; area of the response was 0.1 cm^2) occurred immediately after

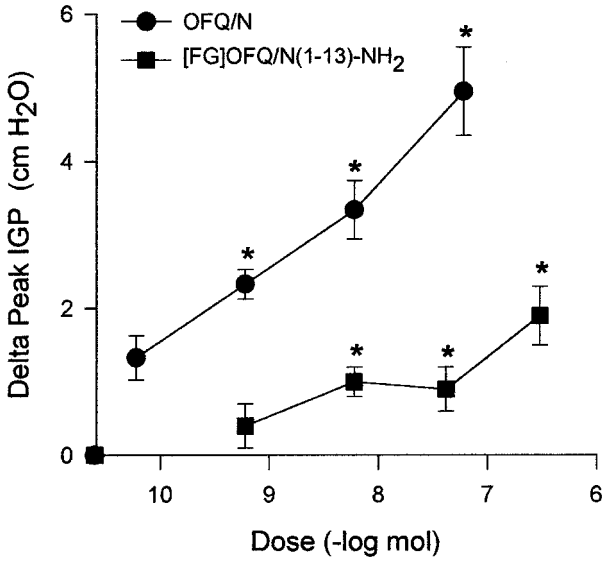

Figure 2 Effects of vehicle and OFQ/N (0.06–60 nmol kg^{-1}) or [FG]OFQ/N(1-13)-NH$_2$ (0.6–300 nmol kg^{-1}), administered as a bolus i.v. injection, on peak change in IGP. Data are mean (bar = s.e.) changes from baseline. *Statistically significant when compared with the effect of vehicle.

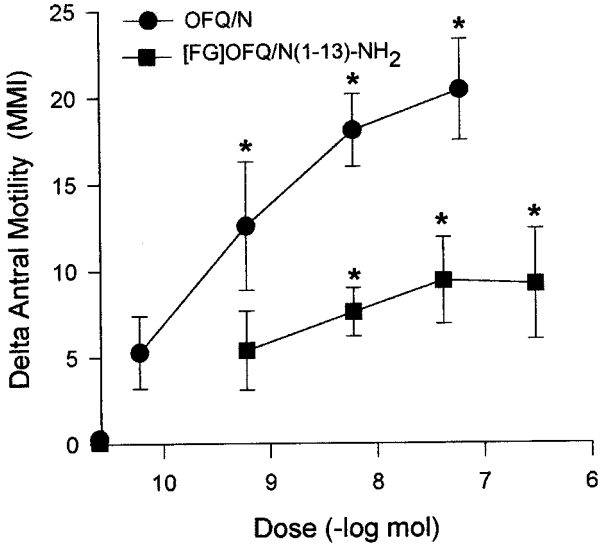

Figure 1 Effects of vehicle and OFQ/N (0.06–60 nmol kg^{-1}) or [FG]OFQ/N(1-13)-NH$_2$ (0.6–300 nmol kg^{-1}), administered as a bolus i.v. injection, on antral motility. Data are mean (bar = s.e.) changes from baseline. *Statistically significant when compared with the effect of vehicle.

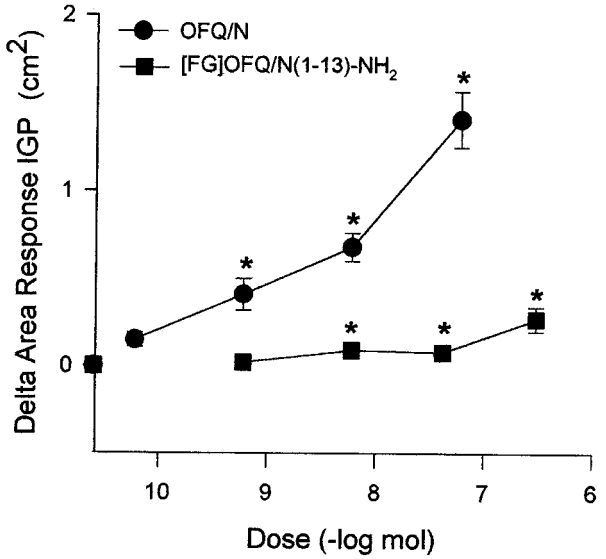

Figure 3 Effects of vehicle and OFQ/N (0.06–60 nmol kg^{-1}) or [FG]OFQ/N(1-13)-NH$_2$ (0.6–300 nmol kg^{-1}), administered as a bolus i.v. injection, on total area of the response IGP. Data are mean (bar = s.e.) changes from baseline. *Statistically significant when compared with the effect of vehicle.

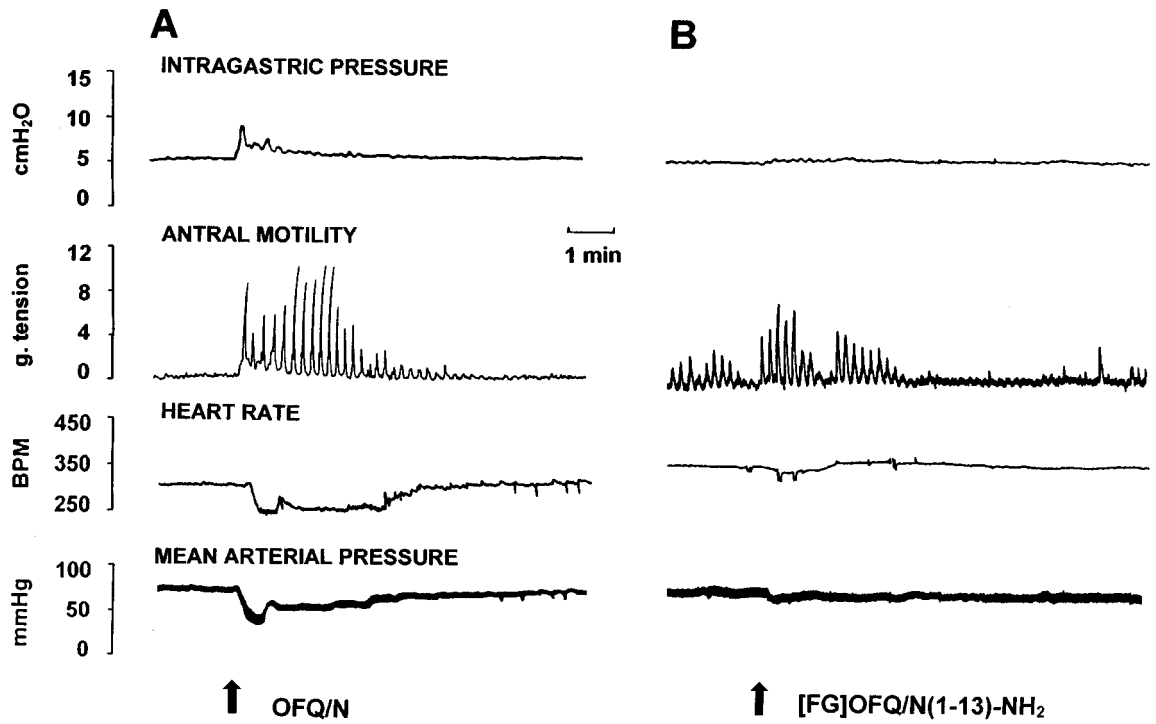

Figure 4 Chart recordings from two different animals injected i.v. with OFQ/N (6 nmol kg^{-1}; (A) or [FG]OFQ/N(1-13)-NH_2 (6 nmol kg^{-1}); (B). Shown are IGP, antral motility, heart rate and mean arterial pressure.

injection and returned to baseline within 3 min. Also shown in Figure 4 are a small decrease in HR and MAP in response to [FG]OFQ/N(1-13)-NH_2. Overall, [FG]OFQ/N(1-13)-NH_2 (0.6–42 nmol kg^{-1}) significantly decreased HR and MAP (data not shown).

The effects of L-NAME, naloxone and [FG]OFQ/N(1-13)-NH_2 on OFQ/N-evoked changes in gastric motor function

The effects of L-NAME (10 mg kg^{-1} i.v.) on gastric motor excitation evoked by OFQ/N are shown in Table 1. When administered i.v. 15 min before a repeat injection of OFQ/N (6 nmol kg^{-1}), L-NAME was unable to alter OFQ/N-evoked increases in antral motility and IGP (Table 1). Nitric oxide synthase inhibition was apparent by the fact that L-NAME alone increased MAP from a baseline of 71 ± 5–131 ± 7 mmHg immediately after L-NAME in these animals.

Naloxone (10 mg kg^{-1}) alone did not affect gastric motor function (Table 2). In addition, when administered i.v. 15 min before repeated injection of OFQ/N (6 nmol kg^{-1}), naloxone was unable to modify the peptide-evoked increases in antral motility and IGP (Table 1).

Administration of OFQ/N (6 nmol kg^{-1}) 15 min after [FG]OFQ/N(1-13)-NH_2 (42 nmol) still resulted in OFQ/N evoked increases in antral motility and IGP. These responses did not differ from those evoked by OFQ/N before the derivative in the same animals (Table 1). Similarly, administration of OFQ/N (6 nmol kg^{-1}) 15 min after the pseudopeptide (42 nmol kg^{-1}) resulted in a hypotension (MAP -26 ± 16 mmHg) that did not differ from that evoked by OFQ/N before the antagonist (MAP -36 ± 11 mmHg) in the same animals.

We were concerned that the lack of antagonist effect of [FG]OFQ/N(1-13)-NH_2 may be due to the dose or the time course of administration of the derivative. Therefore, OFQ/N (6 nmol kg^{-1}) was administered 5, 15 and 30 min after [FG]OFQ/N(1-13)-NH_2 at a dose of 300 nmol kg^{-1} in the same animals (Table 3). Interestingly, when OFQ/N was administered 5 min after [FG]OFQ/N(1-13)-NH_2 the increase in antral contractility and total area of the response of IGP were not significantly different from vehicle administration in the same animals. A small but significant increase in peak IGP was observed (Table 3). Similarly, the marked hypotension in response to OFQ/N was not observed when OFQ/N was administered 5 min after [FG]OFQ/N(1-13)-NH_2. On the other hand, injection of OFQ/N at 30 min after [FG]OFQ/N(1-13)-NH_2 evoked the significant increases in gastric motor excitation and decreases in blood pressure (Table 3). The gastric motor effect of OFQ/N 30 min after the derivative (Table 3), are similar in magnitude to those obtained after the same dose of OFQ/N alone (Figures 1–3).

To assess whether the absence of gastric motor and blood pressure responses to OFQ/N administered 5 min after

Table 1 Gastric motor changes evoked by OFQ/N (6 nmol kg^{-1}) before and 15 min after L-NAME (10 mg kg^{-1}; $n=6$) naloxone (10 mg kg^{-1}; $n=5$), and [FG]OFQ/N(1-13)-NH_2 (42 nmol kg^{-1}; $n=5$)

Treatment	*Antral MMI*	*Peak IGP* (cm H_2O)	*Area response IGP* (cm^2)
Vehicle	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
OFQ/N	14.5 ± 3.9*	3.3 ± 1.0*	0.5 ± 0.1*
L-NAME + OFQ/N	9.6 ± 5.1*	2.3 ± 0.7*	0.3 ± 0.2*
Vehicle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
OFQ/N	19.1 ± 3.7*	1.8 ± 0.5*	0.3 ± 0.1*
Naloxone-OFQ/N	12.6 ± 4.6*	1.3 ± 0.4*	0.2 ± 0.1*
Vehicle	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
OFQ/N	12.6 ± 2.6*	2.4 ± 0.6*	0.3 ± 0.1*
[FG]OFQ/N(1-13)-NH_2 + OFQ/N	11.9 ± 2.8*	1.8 ± 0.5*	0.3 ± 0.1*

*Statistically significant when compared with corresponding mean for vehicle administration.

[FG]OFQ/N(1-13)-NH_2 was due to blockade of the OFQ/N receptor or due to tachyphylaxis, we compared the effects of two repeated injection of OFQ/N (6 nmol kg^{-1}) administered 5 min apart ($n=6$; Table 3). The second administration of OFQ/N failed to elicit changes in either gastric motor function or blood pressure. In addition, the gastric motor and blood pressure responses to the second administration of OFQ/N did not differ from those in response to OFQ/N administered 5 min after [FG]OFQ/N(1-13)-NH_2. These data provide evidence for tachyphylaxis upon rapid repeated administration of OFQ/N.

Effects of vagotomy and spinal cord transection on OFQ/N-evoked changes in gastric motor function

After vagotomy and spinal cord transection, OFQ/N (6 nmol kg^{-1}) still evoked significant increases in antral motility and IGP (both peak and total area of the response; Table 4). Vagotomy or spinal cord transection alone did not alter IGP and antral motility (Table 2).

Discussion

This study shows that peripherally administered OFQ/N potently stimulates gastric motility in anaesthetized rats. The OFQ/N derivative, [FG]OFQ/N(1-13)-NH_2 also increases gastric motor function although with less potency than OFQ/N. Although [FG]OFQ/N(1-13)-NH_2 has been suggested to be an antagonist at the OFQ/N receptor, this derivative was unable to prevent the gastric motor effects produced by OFQ/N, unless given immediately (5 min) prior to the peptide. This effect seems to be related to tachyphylaxis rather than antagonistic properties of the derivative. The gastric effects of the peptide and its derivative are apparently directed toward enhancement of gastric antral contractile activity, with less marked increases in IGP. We will discuss our results in terms of the previous studies on the effects of OFQ/N on the gastrointestinal tract, the possible mechanisms by which OFQ/N may mediate its effects, and the actions of [FG]OFQ/N(1-13)-NH_2 at the OFQ/N receptor in several experimental settings.

Previous studies have demonstrated that OFQ/N markedly stimulates rodent colonic motility when given centrally (Osinski *et al*., 1999a) and systemically (Taniguchi *et al*., 1998; Osinski *et al*., 1999a; Menzies *et al*., 1999). Large intestinal transit is also significantly reduced by OFQ/N (Taniguchi *et al*., 1998; Osinski *et al*., 1999a). These effects are not naloxone reversible and therefore, they are not mediated through classical opioid receptors. This is a concern since all three major types of opioid receptors are present in the gastrointestinal tract and their activation induces circular muscle contractions (Fox-Threlkeld *et al*., 1994; Meunier *et al*., 1995). In addition, OFQ/N displays significant amino acid sequence homology with dynorphin A (Reinscheid *et al*., 1995) and resembles the opioid peptides with respect to the inhibition of adenylyl cyclase (Connor *et al*., 1996). However, OFQ/N differs from the other opioid receptor agonists in that it does not possess the N-terminal tyrosine residue that is essential for activity at opioid receptor subtypes (Varani *et al*., 1999). Since naloxone failed to reverse the effect of OFQ/N on gastric motility, the effects of this peptide are independent of a direct action on opioid receptors.

The gastric motor effects of OFQ/N are mediated neurally, since interference with neural transmission by veratridine, serosal benzalkonium chloride treatment, or tetrodotoxin abolishes the response (Osinski *et al*., 1999a; Yazdani *et al*., 1999). *In vitro* electrical field stimulation studies on colon strips have demonstrated that OFQ/N inhibits 'on' contractions probably through inhibition of cholinergic transmission (Osinski *et al*., 1999a; Yazdani *et al*., 1999). One way in which OFQ/N could increase gastrointestinal motility is through the inhibition of descending enteric nitric oxide synthase pathways to result in motor excitation. However, in our studies nitric oxide synthase inhibition had no effect on the OFQ/N-evoked increases in gastric motility, similar to results in the colon

Table 2 Effects of vagotomy ($n=5$), spinal cord transection (SCT, $n=4$), L-NAME (10 mg kg^{-1}, $n=6$), and naloxone (10 mg kg^{-1}, $n=5$) on baseline antral motility and peak IGP

Treatment	*Antral MMI*	*Peak IGP* (cm H_2O)
Before vagotomy	3.0±1.2	3.3±0.6
45 min after vagotomy	3.7±0.5	4.6±0.7
Before SCT	8.9±4.2	3.6±0.8
60 min after SCT	4.3±2.5	3.1±0.6
Before L-NAME	2.0±0.6	4.0±0.4
15 min L-NAME	1.3±0.8	3.7±0.4
Before naloxone	0.0±0.0	0.0±0.0
15 min after naloxone	2.2±2.2	3.9±0.6

Table 4 Antral motility and IGP changes evoked by OFQ/N (6 nmol kg^{-1}) before and after bilateral cervical vagotomy ($n=5$) or spinal cord transfection (SCT; $n=4$)

Treatment	*Antral MMI*	*Peak IGP* (cm H_2O)	*Area response* (cm_2)
Vehicle	−0.2±0.1	0.0±0.0	0.0±0.0
OFQ/N before vagotomy	12.6±4.3*	3.7±0.7*	0.6±0.1*
OFG/N after vagotomy	12.4±4.4*	3.2±0.5*	0.9±0.2*
Vehicle	−0.8±0.5	0.0±0.0	0.0±0.0
OFQ/N before SCT	12.8±5.3*	3.6±1.2*	0.7±0.1*
OFQ/N after SCT	11.6±5.5*	2.9±0.6*	0.7±0.2*

*Statistically significant when compared with corresponding mean for vehicle administration.

Table 3 Effects of OFQ/N (6 nmol kg^{-1}) administered i.v. at different time intervals after [FG]OFQ/N(1-13)-NH_2 (300 nmol kg^{-1}) on antral motility and IGP in six rats

Treatment	*Antral MMI*	*Peak IGP* (cm H_2O)	*Area response IGP* (cm^2)	*MAP* (mmHg)
Vehicle	0.0±0.0	0.0±0.0	0.0±0.0	0±1
OFQ/N 5 min after [FG]OFQ/N(1-13)-NH_2	4.8±2.4	0.8±0.1*†	0.1±0.0†	−1±1†
OFQ/N 15 min after [FG]OFQ/N(1-13)-NH_2	7.8±3.0*	1.3±0.2*	0.2±0.1*	−8±3
OFQ/N 30 min after [FG]OFQ/N(1-13)-NH_2	10.3±3.5*	1.7±0.2*	0.3±0.1*	−15±5*
OFQ/N 5 min after OFQ/N	3.4±1.0†	0.8±0.1†	0.1±0.0†	−3±1†

*Statistically significant when compared with corresponding mean for vehicle administration. †Statistically significant when compared with OFQ/N 30 min after [FG]OFQ/N(1-13)-NH_2.

(Yazdani *et al*., 1999). The latter investigators performed a rigorous pharmacological approach to determine the pathway by which OFQ/N increased colonic motility; however, the mechanisms remain elusive. The present study suggests that OFQ/N mediates gastric motor excitation in a manner similar to how it effects colonic motility, but the actual pathway responsible for this effect also remains unknown. It is unlikely that the gastric motor effects are secondary to the hypotension observed in this and previous studies (Giuliani *et al*., 1997) (Champion & Kadowitz, 1997). Our previous studies have shown that gastric motor changes can be evoked independently of systemic blood pressure decreases. For example, systemically administered delta^9-tetrahydrocannabinol in rats causes a prolonged hypotension, but in this case the gastric motor activity is decreased (Krowicki *et al*., 1999). Thus, increased gastric motor activity is unlikely to be causally related to hypotension.

Our results are in conflict with those of a previous study (Yazdani *et al*., 1999) demonstrating that OFQ/N had no effect on gastric muscle strips *in vitro*. The differences in our results could be related to differences in the experimental preparations. We hypothesized that OFQ/N increases contractility *in vivo* due to actions on descending vagal or sympathetic pathways to the stomach. However, in our studies vagotomy or spinal cord transection did not prevent the OFQ/N mediated increase in gastric motility. Since neither intact descending spinal sympathetic pathways nor vagus nerves are essential for the OFQ/N-mediated response, the peptide presumably acts at the level of the enteric nerves. This being the case, we have no explanation for the apparent discrepancy between our results and those of Yazdani *et al*. (1999). However, it should be noted that, in the latter study, only one figure of an individual case was presented to demonstrate the absence of OFQ/N effects in gastric muscle strips. Since no compiled data or statistical analysis were presented, it is hard to assess how representative this example was of their study. Indeed, centrally administered OFQ/N decreased upper gastrointestinal transit time (Osinski *et al*., 1999a). Although the method used in this study could not discriminate between changes in gastric emptying and small intestinal transit, these and the present results show that there is no reason to exclude the stomach as a site of action of the pro-motility effects of OFQ/N. Indeed, RT – PCR detected OFQ/N receptor message throughout the pig gastrointestinal tract (Osinski *et al*., 1999b). A preliminary study illustrated different splice variants of the OFQ/N gene in different regions of the GI tract. However, in light of the present study, this is unlikely to result in tissue heterogeneity of the response, as was suggested (Curro *et al*., 1999).

Both OFQ/N and [FG]OFQ/N(1-13)-NH_2 increased colonic motility in rats and mice (Sann *et al*., 1999; Menzies *et al*., 1999). The [FG]OFQ/N(1-13)-NH_2 analogue had approximately 100 times lower potency than OFQ/N to contract colonic strips *in vitro* (Menzies *et al*., 1999). Preliminary *in vivo* data show that [FG]OFQ/N(1-13)-NH_2 acted with a 10 fold lower potency than OFQ/N (Sann *et al*., 1999). Our results obtained in stomach tissue are in complete agreement with these observations obtained in colonic tissue. However, since [FG]OFQ/N(1-13)-NH_2 has been reported to act as a selective antagonist of the OFQ/N receptor in the guinea-pig ileum and mouse vas deferens *in vitro* (Guerrini *et al*., 1998), we tried several different paradigms to reveal potential antagonism at the OFQ/N receptor. In this respect we initially chose a dose of 42 nmol kg^{-1} [FG]OFQ/N(1-13)-NH_2. This was based on the fact that at least 10 times higher of a concentration of the OFQ/N derivative is required to evoke a rightward shift of the concentration-response curve to OFQ/N in guinea-pig ileum preparation *in vitro* (Guerrini *et al*., 1998). In a later study, these investigators showed that [FG]OFQ/N(1-13)-NH_2 can act as a full agonist to inhibit stimulated cyclic AMP accumulation in Chinese hamster ovary cells transfected with the OFQ/N receptor (Okawa *et al*., 1999). It was concluded that [FG]OFQ/N(1-13)-NH_2 can act as a partial agonist or an antagonist in different situations (Guerrini *et al*., 1998; Okawa *et al*., 1999). Therefore, in the present study, we also tested a 50 times higher dose of [FG]OFQ/N(1-13)-NH_2 in an attempt to block the excitatory gastric motor effects of OFQ/N. When administered alone, [FG]OFQ/N(1-13)-NH_2 (42 and 300 nmol kg^{-1}) produced a similar pattern of gastric motor responses. In addition, the gastric motor effects of OFQ/N were only prevented when this peptide was administered 5 min after the injection of [FG]OFQ/N(1-13)-NH_2. Analogous to this latter observation, the gastric motor responses to repeated administration of OFQ/N were shown to be abolished and similar to vehicle control when OFQ/N injections were made 5 min apart. Together, these findings suggest that the antagonist properties of [FG]OFQ/N(1-13)-NH_2 observed in our studies may be due to tachyphylaxis rather than to blockade of the OFQ/N receptor. Therefore, factors such as the tissue OFQ/N receptor reserve may determine the extent to which [FG]OFQ/N(1-13)-NH_2 exerts antagonistic behaviour in different experimental settings.

Although not a focus of the present studies, we also considered the effects of the antagonist on cardiovascular function. OFQ/N evoked similar dose-related decreases in MAP as that reported in previous studies (Czapla *et al*., 1997; Giuliani *et al*., 1997; Kapusta & Kenigs 1999; Kapusta *et al*., 1999a, b). It has been reported that there is a marked reduction in the hypotensive response to OFQ/N when it is administered 5 min after 300 nmol kg^{-1} [FG]OFQ/N(1-13)-NH_2 in rats (Bigoni *et al*., 1999). Similarly, the OFQ/N-evoked hypotensive response in conscious mice was reduced when the OFQ/N was administered 5 min after pseudopeptide (Madeddu *et al*., 1999). In our study, the hypotension evoked by OFQ/N 5 min after OFQ/N was similar to that after the pseudopeptide and did not differ from the response to vehicle. Thus, both the apparent reversal of both the OFQ/N-evoked hypotension and gastric motor increases by the pseudopeptide may be due to tachyphylaxis rather than pharmacological blockade of the OFQ/N receptor.

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