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Cloning, expression and functional role of a nociceptin/orphanin FQ receptor in the porcine gastrointestinal tract

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Abstract

The heptadecapeptide nociceptin/orphanin FQ is the cognate ligand for the opioid receptor-like orphanin FQ (OFQ) receptor, a member of the G protein-coupled receptor superfamily. The gastrointestinal tract is a major site of opioid action, and preliminary evidence suggests that an OFQ receptor may be expressed in rat small intestine. We addressed the hypothesis that this receptor is expressed in the gastrointestinal tract of the pig, a model for the human digestive system. A 1205-bp cDNA was isolated from porcine forebrain which contained the 370 amino acid open reading frame encoding the OFQ receptor. The receptor mRNA is likely to arise from a single gene, as determined by Southern blotting of porcine genomic DNA restriction digests using a porcine OFQ receptor cDNA probe. A semi-nested reverse transcriptase–polymerase chain reaction survey of receptor mRNA indicates that it is expressed in the porcine cerebral cortex and kidney, and along the length of the gastrointestinal tract. OFQ decreased initial contractile responses of porcine ileal smooth muscle strips to trains of electrical field stimulation with an IC_{50} value of 1.3 nM; its effects were resistant to the opioid antagonist, naloxone. The peptide, at concentrations $\geq$ 3 nM, also attenuated I_{sc} elevations evoked by electrical transmural stimulation of mucosa–submucosa sheets from porcine ileum. The actions of OFQ appeared to differ from those previously reported for opioid receptor agonists in these tissue preparations. These results indicate that an OFQ receptor is expressed in the porcine intestine which modulates the neural control of intestinal smooth muscle contractility and mucosal transport.

Keywords: Myenteric plexus; 'Orphan' opioid receptor; Species homology; mRNA expression

1. Introduction

An opioid receptor-like orphan receptor was discovered through low stringency hybridization screening of a human brainstem cDNA library using probes synthesized by RT-PCR with degenerate primers to the conserved regions of the three opioid receptor cDNAs (Mollereau et al., 1994) or after screening a rat cerebrum cDNA library with a mouse δ-opioid receptor probe (Fukuda et al., 1994). The novel receptor is a member of the G protein-coupled receptor superfamily and manifests high (> 60%) sequence identity with the three (μ, δ and κ) cloned opioid receptors. A heptadecapeptide ligand for this receptor has been isolated from rat and porcine brain and termed nociceptin or orphanin FQ (OFQ; Meunier et al., 1995; Reinscheid et al., 1995). Like opioid receptors, orphanin FQ receptor activation has been linked to the suppression of adenylyl cyclase activity and modulation of neuronal K^+ and Ca^{2+} conductances. However, receptors mediating the effects of OFQ and opiates manifest different pharmacological characteristics (Meunier, 1997). OFQ and its receptor have been shown to be expressed widely throughout the central nervous system, and they may be involved in such functions as pain neurotransmission, opioid tolerance development, motivated behaviors, learning, auditory signal processing and neuronal differentiation (Darland et al., 1998).

The gastrointestinal tract is a major site of opiate action; opiates have throughout history been known to alleviate diarrhea and produce constipation (Kromer, 1988). Opioid receptor agonists acting through one or more of the three cloned opioid receptors alter smooth muscle contractions and modulate epithelial ion transport in the small intestine. These receptors are expressed on enteric neurons and smooth muscle cells (Creese and Snyder, 1975; Bitar and

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Makhlouf, 1985). Despite the similarities between OFQ and opioid receptors, few studies have examined the expression and role of the OFQ receptor in the gastrointestinal tract. In situ hybridization and Northern blot analyses of mRNA encoding the OFQ precursor protein suggest that there is little or no expression of OFQ in the intestinal tract (Mollereau et al., 1996; Nothacker et al., 1996). However, these approaches may not be sufficiently sensitive to detect prepro-OFQ mRNA which may exist in low abundance in this tissue. Indeed, it has been only through the deployment of relatively more sensitive reverse transcriptase-polymerase chain reaction (RT-PCR) techniques that a low abundance message for the OFQ receptor could be detected in rat intestine (Wang et al., 1994).

In the present investigation, we addressed the hypothesis that the OFQ receptor is expressed in the gastrointestinal tract of the pig, which we use as an animal model for the study of opiates on human intestinal function (Brown et al., 1996). To this end, we report the isolation of the complete cDNA sequence of the porcine OFQ receptor and the RT-PCR-based detection of receptor mRNA expression in the porcine gut and other peripheral tissues. Furthermore, we demonstrate that OFQ modulates contractility and ion transport in isolated smooth muscle and mucosa, respectively, from the porcine ileum, actions which are presumably mediated by intestinal OFQ receptors.

2. Materials and methods

2.1. Animals

Yorkshire pigs (6–10 weeks old) of either sex were obtained from the University of Minnesota Swine facility (Rosemount, MN). They were provided with standard feed (Rosemount Feed Mill, Rosemount, MN) ad libitum. They were anesthetized with a tiletamine hydrochloride–zolazepam combination (Telazol®; 0.5 mg/lb, i.m.; Fort Dodge Laboratories, Fort Dodge, IA). They were subsequently euthanized with pentobarbital (Beuthanasia-D®, 30 mg/lb, i.v., Schering-Plough Animal Health, Kenilworth, NJ) in accordance with the approved University of Minnesota Animal Care Committee protocol number 9505006. A midline laparotomy incision was made and tissues of interest were quickly removed for molecular biological and/or in vitro functional experiments.

2.2. RNA isolation

Total cellular RNA was isolated from freshly obtained tissues with acid guanidium–phenol–chloroform extraction (Chomczynski and Sacchi, 1987) and subsequently treated with 5 units of RQ1 RNase-Free DNase (Promega, Madison, WI) for 15 min at 37°C. After an additional phenol/chloroform extraction and ethanol/sodium acetate precipitation, RNA was dissolved in ribonuclease-free water and quantified with UV spectrophotometry. Additionally, RNA integrity was verified with denaturing agarose gel electrophoresis.

2.3. cDNA synthesis

One to two μg of total RNA were preincubated with 100 ng of random hexamer primers for 10 min at 70°C. RNA was reverse transcribed in a final volume of 20 μl containing 10 units of RNasin Ribonuclease Inhibitor (Promega), 200 units of Moloney murine leukemia virus reverse transcriptase (SuperScript II; Life Technologies, Gaithersburg, MD), 10 mM dithiothreitol, 1 × First Strand buffer and 1 mM of each deoxynucleotide triphosphate (Pharmacia, Piscataway, NJ). The RT reactions were incubated for 10 min at room temperature and then for 50 min at 42°C. Reactions were stopped by heating the samples at 70°C for 15 min. Two units of ribonuclease H (Life Technologies) were added to each sample followed by incubation for 30 min at 37°C. Reactions were terminated by heating at 70°C for 10 min and were stored at 4°C until needed. Successful cDNA synthesis was determined by PCR amplification of β-actin message.

2.4. Orphanin FQ receptor mRNA isolation

Two oligonucleotide primers based on the human OFQ receptor sequence were designed for PCR amplification of an 880 base pair (bp) fragment of OFQ-R from porcine forebrain cDNA. The sequences of the primers were: 5′-CATCTGCTGCTCAATGCC-3′ and 5′-CTTGAAGTTCTCATCCAGGAA-3′. The resulting amplification product was sequenced on an Applied Biosystems Model 377 automated fluorescence sequencer. The 5′ and 3′ ends of the OFQ receptor were cloned by RACE-PCR (Frohman, 1994). Finally, a pair of PCR primers were designed to amplify the entire receptor open reading frame from brain and peripheral tissues. Primer sequences were: 5′-GGGAACAGAATGCCAGCAG-3′ and 5′-TCCACGCCTAGTCATGCAG-3′. PCR was catalyzed with the Expand Long Template System (Boehringer-Mannheim; Indianapolis, IN) to insure high fidelity DNA synthesis. Reactions commenced with an initial two min denaturation period at 95°C prior to introduction of the thermostable DNA polymerase to the reaction tubes. After addition of polymerase, ten cycles of 1 min at 94°C, 1 min at 62°C, 2 min at 68°C were performed followed by an additional 20 cycles with the same parameters but with each successive 68°C extension cycle increased by 20 s. At the conclusion of the thirty cycles, a seven min final extension was performed at 68°C. PCR was conducted with a Model 480 DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, CT). One-tenth of the reactions were electrophoresed through a 2% agarose gel; products of interest were excised from the gel and eluted with the QiaQuick Gel Extraction kit

(Qiagen; Valencia, CA). Purified products were ligated into pT7 Blue-2 T-vector (Novagen; Madison, WI) and multiple clones were sequenced on both strands. Sequence analyses were performed with the Wisconsin Package v. 8.0 (GCG; Madison, WI).

2.5. Southern blotting

DNA was isolated from porcine peripheral blood with the Puregene DNA isolation kit (Gentra Systems; Minneapolis, MN). Restriction digestion reactions containing 15 μg of genomic DNA were performed with *Bam*HI (Boehringer-Mannheim Biochemicals), *Eco*RI (Boehringer-Mannheim Biochemicals), and *Bbs*I (New England BioLabs; Beverly, MA). Reactions were incubated at 37°C overnight and then electrophoresed through a 0.7% agarose gel prior to overnight capillary transfer in 20× saline-sodium citrate to a positively charged nylon membrane (Boehringer-Mannheim Biochemicals). Filter hybridization was carried out with an 880 bp digoxigenin labeled cDNA probe for OFQ receptor according to the manufacturer's protocol (DIG/Genius System User's Guide for Membrane Hybridization v. 3.0; Boehringer-Mannheim Biochemicals). Hybridization bands were detected with chemiluminescence and captured on X-ray film.

2.6. Tissue survey of OFQ receptor mRNA expression

A semi-nested RT-PCR assay was designed to amplify OFQ receptor message from a variety of peripheral tissues. Primers were designed to span at least one intron (based on the genomic structure of the mouse OFQ receptor gene). This allowed PCR products to be readily distinguished from the possible amplification of contaminating DNA. An initial round of PCR was conducted for 40 cycles (94°C for 1 min; 59°C for 1 min; 72°C for 1 min). Amplicons from this first round were diluted 1:100 and 1 μl was used in a second PCR of 20 additional cycles of the same parameters. Reaction products were electrophoresed through 2% agarose gels and visualized with ethidium bromide staining. Reaction products were sequenced to confirm their identity as OFQ receptor partial transcripts.

2.7. Measurement of smooth muscle contractility

A portion of the ileum was excised and placed in ice-cold, oxygenated physiological salt solution, modified to approximate the composition of porcine extracellular fluid (composition in mM: Na^+, 149; K^+, 6; Cl^-, 140; Mg^{2+}, 0.7; Ca^{2+}, 3; HCO_3^-, 20; HPO_4^{2-}, 1.3; and $H_2PO_4^-$, 0.3; and D-glucose, 11; pH 7.4). The ileal segment was cut longitudinally opposite its mesenteric attachment and placed in oxygenated physiological salt solution at 39°C (porcine core temperature). Ileal segments were pinned out as a flap, with the mucosa uppermost. Both the mucosa and submucosa were removed and a 3 mm × 10 mm muscle strip was cut parallel to the circular muscle layer, around the entire circumference of the ileum. The strip therefore contained the circular muscle, from which isometric recordings were made, as well as the myenteric plexus and the longitudinal smooth muscle.

Circularly oriented, mucosa-free muscle strips were mounted in 15 ml organ baths containing the porcine-modified physiological salt solution aerated with 95% O_2 and 5% CO_2 at 39°C. The preparation was mounted under an initial tension of 9.8 mN. Mechanical activity was recorded isometrically by a strain-gauge transducer (Grass Model FT03, Astro-Med Grass, West Warwick, RI) connected to a Grass Model 79D polygraph. Strips were equilibrated for 60 min and the bath medium was changed every 15 min. After spontaneous muscle activity stabilized, L_o (i.e., the length at which contraction amplitude was a maximum) was determined in order to maintain a standard maximum contraction in every tissue. Electrical field stimulation was applied to each muscle strip through a pair of platinum ring electrodes connected to the output of Grass S88 dual-channel stimulator. The electrodes were shaped into loops of 3 mm diameter and were separated from each other by 3 mm. The muscle strips were passed through the loops and pulses of 1 ms duration were delivered at a supramaximal amplitude of 70 V at 10 Hz in 10 s trains every 100 s. Field-stimulated contractions were eliminated by 100 nM saxitoxin (data not shown).

Measurements of OFQ actions on stimulation-induced contractions were made in separate muscle strips after electrical field stimulation was shown to produce contractions of consistent amplitude. After electrically evoked contractions remained stable for 3 successive stimulation periods, the effects of OFQ (nociceptin; Peninsula Laboratories, Belmont, CA) on contraction amplitude were measured at increasing concentrations ranging from 0.3–100 nM. In some experiments, tissues were pretreated with 1 μM naloxone prior to OFQ addition.

2.8. Measurement of mucosal ion transport

The porcine ileal submucosa–mucosa was mounted between two lucite Ussing-type half chambers having a flux area of 0.95 cm^2. Mucosal sheets were bathed in the porcine-modified physiological salt solution, maintained at pH 7.4 at 39°C and continuously oxygenated (5% CO_2 in O_2) by gas lift. Bathing media were contained in 10 ml water-jacketed glass reservoirs. D-Glucose and mannitol were added to the media bathing the serosal and luminal sides, respectively, of each sheet to achieve final concentrations of 10 mM. Short-circuit current (I_{sc}) was measured continuously under voltage-clamped conditions as previously described (Hildebrand and Brown, 1990); open-circuit transepithelial potential difference was measured at brief time intervals and tissue conductance was calculated by Ohm's law. Rectangular, bipolar pulses of electric current (300 pulses at 10 Hz, 0.5 ms pulse dura-

tion, 30 V/cm^2) were delivered across submucosa-mucosa sheets. Peak increments in I_{sc} relative to baseline values were determined in response to electrical transmural stimulation after stabilization of the spontaneous I_{sc}. After the electrically evoked I_{sc} returned to basal levels (approximately 5 min after the stimulus was terminated), OFQ was administered in cumulatively increasing concentrations (3–30 nM) to the serosal aspect of each tissue. An electrically evoked mucosal response was produced after each OFQ treatment and compared to the initial (control) response to electrical transmural stimulation determined in the absence of the peptide.

2.9. Data analysis

The peak contraction amplitude of smooth muscle strips was measured from baseline values in mN tension. The average of 3 successive contractions occurring after application of each OFQ concentration was determined. OFQ-mediated inhibition of stimulation-induced circular muscle contractions was expressed as the mean percentage of inhibition of pre-peptide contractions. The concentration of OFQ producing 50% inhibition of the twitch response (IC_{50}) was determined using the PRISM software program (GraphPad Software, San Diego, CA). Comparisons be-

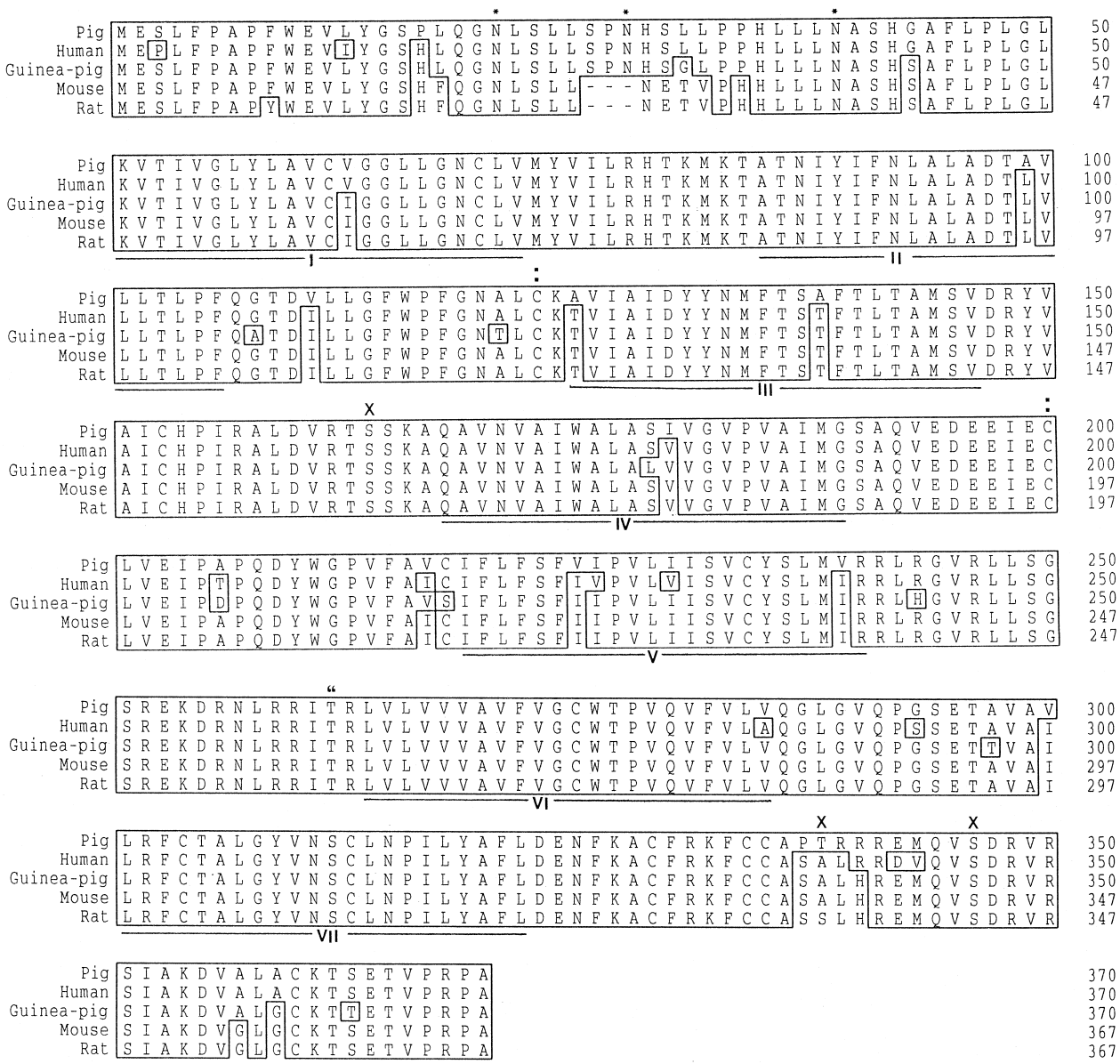

Fig. 1. Box plot of amino acid sequence alignment of porcine OFQ receptor with its human (GenBank accession number X77130), guinea-pig (U04369), mouse (U04952), and rat (U01913) homologs. The transmembrane spanning domains are underlined and numbered I through VII. Symbols: (—), gap introduced for sequence alignment; (:), conserved cysteine residues predicted to form disulfide bonds; (∗), potential N-glycosylation site; ("), potential cAMP- and cGMP-dependent protein kinase phosphorylation site; (×), potential protein kinase C phosphorylation sites. The sequence for the porcine receptor has been submitted to GenBank (accession number U72758).

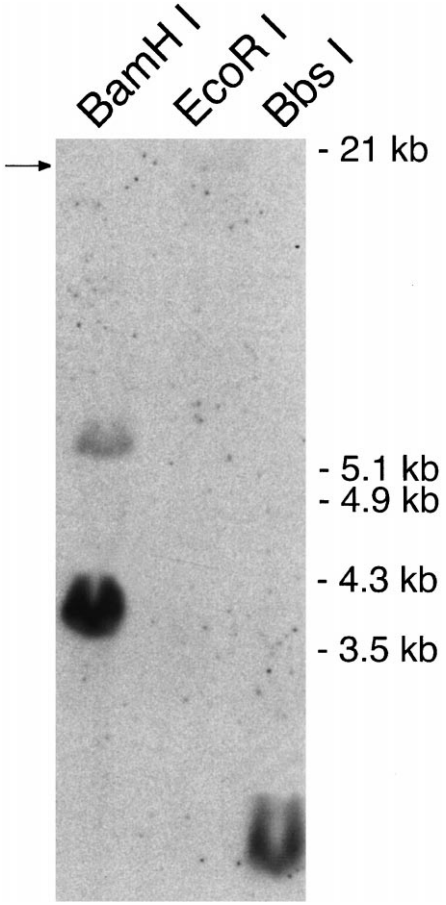

Fig. 2. Southern blot of 15 μg of porcine genomic DNA digested overnight with indicated restriction enzymes followed by hybridization with a digoxigenin-labeled 880 bp porcine OFQ receptor fragment and chemiluminescent detection. Arrow indicates faint band present in the lane containing *Eco*RI-digested DNA.

tween mean mucosal responses to electrical field stimulation before and after OFQ treatment were made with a one-way analysis of variance followed by Dunnett's multiple comparison test with the limit for statistical significance set at $P < 0.05$.

3. Results

3.1. Porcine OFQ receptor cDNA isolation

A 1205 bp cDNA which contained the 370 amino acid open reading frame encoding the OFQ receptor was isolated from porcine forebrain (Fig. 1). Porcine OFQ receptor manifested 92–94% sequence identity with the human, guinea-pig, rat and mouse ORL_1 (OFQ) receptors. The OFQ receptor protein contains the putative seven transmembrane spanning domains that are characteristic of the G protein-coupled receptor superfamily. Residues that may be targets for postranslational modification include: Asn^{21}, Asn^{28}, and Asn^{39} (potential *N*-glycosylation sites); Cys^{123} and Cys^{200} (putative disulfide bond conserved among opioid receptors); Thr^{262} (potential cAMP- and cGMP-dependent protein kinase phosphorylation site); and, Ser^{164}, Thr^{338}, and Ser^{346} (potential protein kinase C phosphorylation sites).

3.2. Southern blotting

To determine if the multiple RNA species were the products of one or multiple genes, Southern blot analysis was performed. *Bam*HI, *Bbs*I, and *Eco*RI were all predicted to cleave only once based on the cDNA sequence.

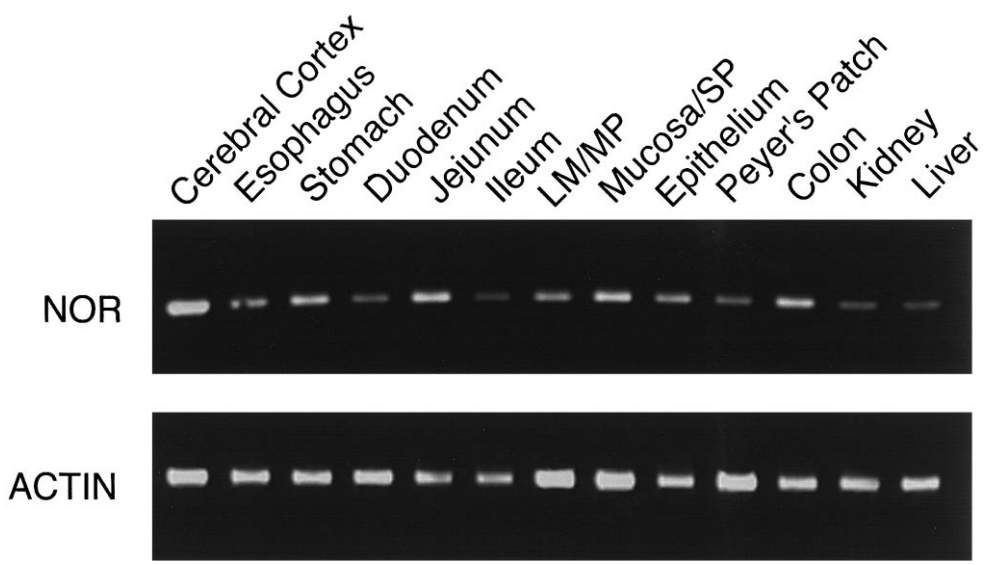

Fig. 3. Porcine OFQ receptor (as nociceptin receptor, NOR) amplification products resulting from the semi-nested RT-PCR assay in cerebral cortex, regions of the gastrointestinal tract, subregions in the wall of the ileum, kidney and liver. All products represented the porcine OFQ receptor as determined by automated DNA sequencing. RT-PCR amplification of actin message was included as a positive control. The ileal epithelium consisted of epithelial cells and intraepithelial nerve fibers, but lacked submucosal nerve cell bodies. The ileal Peyer's patch is a continuous band of organized lymphoid tissue present in porcine ileum. Abbreviations for ileal subregions: LM/MP, longitudinal muscle-myenteric plexus; and SP, submucosal plexuses.

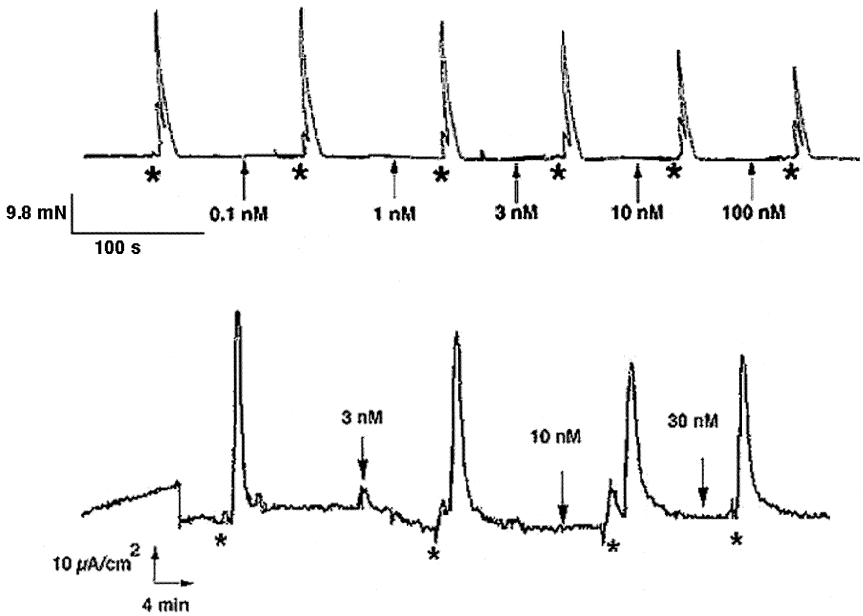

Fig. 4. Top: Representative contractile response of porcine ileum to electrical field stimulation. OFQ given at increasing concentrations reduced electrically-evoked 'on' responses of this preparation. Tracing is representative of experiments performed in 9 tissues from 4 pigs; 1 g tension = 9.8 mN. Bottom: Representative mucosal I_{sc} response of porcine ileum to transmural electrical stimulation (ETS). At 3 nM, serosal OFQ alone produced a small decrease in baseline Isc of 3.0 ± 0.8 $\mu A/cm^2$ ($n = 1$ tissue from each of 5 pigs). Electrical transmural stimulation (denoted by asterisks) produced transient increases in I_{sc} which were reduced by OFQ at concentrations $\geqslant 3$ nM (at arrows).

The banding pattern displayed in Fig. 2 indicates that porcine OFQ receptor exists in the porcine genome as a single copy gene.

3.3. Expression and functions of the porcine OFQ receptor in the intestinal tract

Semi-nested RT-PCR assays of porcine OFQ receptor mRNA revealed that it is expressed throughout the intestinal tract, and is not consistently present in the liver (Fig. 3). Because the assay was not quantitative, we could not determine which tissues had higher levels of receptor transcripts. Nevertheless, the widespread distribution of the OFQ receptor in the gut suggests that it may mediate aspects of intestinal function.

To examine the functions of OFQ in the porcine ileum, we first examined its actions on neurogenic contractile responses of smooth muscle strips with attached myenteric

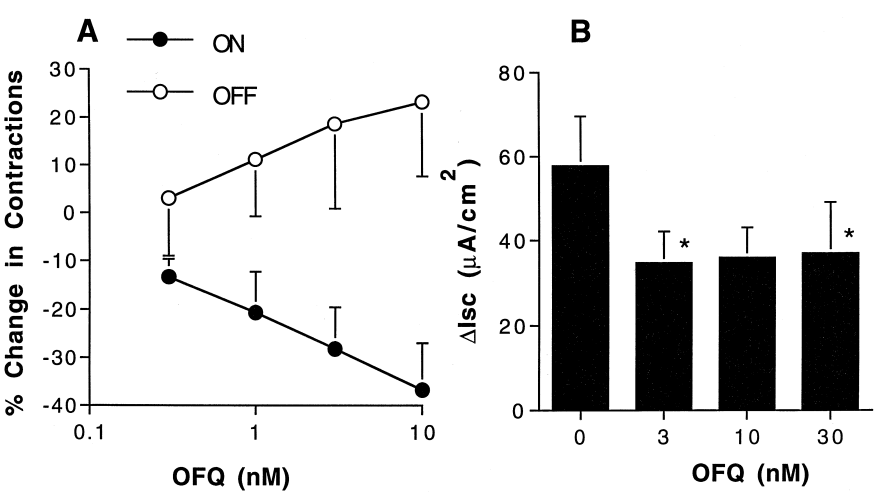

Fig. 5. (A) Reduction in electrically-evoked isometric contractions in circularly oriented ileal smooth muscle by OFQ. Each point represents the mean ± S.E.M. of responses measured in 4 tissues from 4 pigs. (B) Reduction in electrically evoked changes in I_{sc} of mucosal sheet preparations by OFQ. OFQ significantly reduced I_{sc} elevations in response to electrical stimulation at concentrations of 3 and 30 nM, but not at 10 nM ($P < 0.05$, One-way analysis of variance with Dunnett multiple comparison test). Each point represents the mean ± S.E.M. of responses measured in 4 tissues from 4 pigs.

plexus. A representative chart record depicting the effects of electrical field stimulation on smooth muscle strips oriented in the plane of the circular muscle in the absence and presence of OFQ is shown in Fig. 4 (top record). The muscle rapidly contracted at the start of electrical field stimulation, attained maximum amplitude midway through the duration of stimulation and then the contraction began to decay. We refer to this contraction as the 'on' response. At the cessation of stimulation, a rapid rebound contraction ensued. This contraction, which was always larger in amplitude than the 'on' response, is referred to as an 'off' response. We have previously determined that the 'on' response is partially mediated by acetylcholine and the 'off' response is mediated in part by both acetylcholine and substance P (Brown et al., 1998). OFQ produced a concentration-dependent inhibition and potentiation of electrically evoked 'on' and 'off' contractions, respectively; however, its effects on 'off' responses were small and quite variable (Fig. 4, top). The peptide maximally reduced electrically evoked 'on' contractions by $38 \pm 2\%$ relative to pre-OFQ responses with an IC_{50} value of 1.3 nM (95% confidence limits of 0.2–8.3 nM; Fig. 5A). The inhibitory effects of OFQ were not altered in tissues pretreated with the opioid antagonist naloxone at 1 μM (OFQ $IC_{50} = 3.0$ nM, 95% confidence limits: 1.3–7.1 nM; maximum inhibition $= 37 \pm 1\%$; $n = 4$–6 tissues from > 3 pigs).

Second, we assessed the actions of OFQ on ion transport across the porcine ileal mucosa; we have previously determined that active transport in this tissue is under neuromodulatory control (Hildebrand and Brown, 1990). At a serosal concentration of 3 nM, OFQ produced a slight decrease in basal short circuit current (I_{sc}) across sheets of mucosa-submucosa (Fig. 4, bottom record) and slightly increased tissue conductance from 9.1 ± 3.0 to 11.9 ± 2.1 mS/cm^2 ($P > 0.05$, paired t-test, $n = 5$ tissues). At serosal concentrations > 3 nM, OFQ attenuated the rapid increase in I_{sc} evoked by electrical transmural stimulation (Fig. 5B).

4. Discussion

We have successfully cloned from the porcine forebrain and the distal small intestine a full length cDNA which encodes the putative porcine OFQ receptor. As with the cloned opioid receptors, the porcine OFQ receptor exhibits high ($> 90\%$) sequence similarity with its homologs in other mammalian species, particularly the human OFQ receptor. Receptor message was detected throughout the porcine digestive tract by RT-PCR. The expression of receptor message in the porcine esophagus and kidney, and a corresponding lack of mRNA expression in the liver is directly opposite to the previous results of Wang et al. (1994) who also employed a RT-PCR strategy to detect signals for OFQ-R mRNA in rat tissues. These discrepancies might be attributable to species differences in the tissue-specific expression of OFQ-R message or differences in the sensitivity of the RT-PCR technique. In addition to its expression along the length of the intestinal tract, OFQ receptor mRNA was expressed in all subregions of the ileum. The presence of receptor message in ileal Peyer's patches is consistent with previous reports of its expression in other lymphoid cells and tissues, including the spleen (Wang et al., 1994; Halford et al., 1995; Wick et al., 1995; Peluso et al., 1998). The receptor mRNA is likely to arise from a single gene, as determined by Southern blotting of porcine genomic DNA restriction digests using an OFQ receptor cDNA probe. OFQ receptors may be expressed by neurons and immunocytes lying within the intestinal wall.

In strips of porcine ileal smooth muscle with attached myenteric plexus which were oriented in the plane of the circular muscle, OFQ reduced the 'on' contractile response to trains of electrical field stimulation delivered every 100 s. Its actions differed from those of receptor-selective opioid receptor agonists in this tissue preparation. Agonists acting at μ-opioid receptors are ineffective in reducing either contractile response to EFS, and agonists acting selectively at κ- or δ-opioid receptors preferentially decrease 'on' and 'off' responses (Brown et al., 1998). Although both OFQ and the κ-opioid receptor agonist U-50,488 decrease electrically evoked 'on' contractions, only the effects of OFQ are resistant to naloxone. As 'on' and 'off' responses are sensitive to the muscarinic cholinergic antagonist atropine, we hypothesize that OFQ may inhibit acetylcholine release from porcine myenteric neurons. OFQ has been reported to reduce electrically evoked contractions of the guinea pig ileum with EC_{50} values ranging between 3–10 nM, concentrations similar to those used in the present study. In this concentration range, OFQ appears to inhibit enkephalin release from myenteric neurons evoked by electrical field stimulation (Calò et al., 1997; Gintzler et al., 1997; Zhang et al., 1997; Nicholson et al., 1998). OFQ also increases spontaneous contractions of the mouse and rat isolated colon at slightly higher (1–100 nM) concentrations (Yazdani et al., 1997; Taniguchi et al., 1998; Osinski et al., 1998). The different effects of OFQ observed in isolated intestinal preparations might be attributable to differences in the region of the intestinal tract examined, the types of enteric neurons expressing OFQ receptors or the species used as an experimental model.

In the present study, OFQ additionally reduced I_{sc} elevations evoked by electrical transmural stimulation across sheets of mucosa-submucosa from porcine ileum. Electrically evoked elevations in I_{sc} were previously found to be attributable to active chloride secretion and were abolished by tetrodotoxin or ω-conotoxin GVIA (Hildebrand and Brown, 1990). At the concentrations of the peptide employed, the effects of OFQ did not appear to be concentration-dependent. This phenomenon may be due

to the high starting concentrations of OFQ employed, which may have produced a maximal inhibition of the I_{sc} at concentrations $\geqslant 3$ nM. Alternatively, the mucosal effects of OFQ may undergo tachyphylaxis which might complicate the determination of cumulative concentration–effect relationships. OFQ also produced a small decrease in basal I_{sc} which may have been due to the inhibition of neuromodulatory tone. OFQ appears to be less effective than opioid receptor agonists in decreasing basal or electrically evoked I_{sc} in this tissue preparation (Quito and Brown, 1991).

The results of the present study indicate that OFQ receptors are expressed in the porcine intestine and appear to inhibit the neural control of intestinal smooth muscle contractility and mucosal transport. OFQ receptor mRNA is also expressed in porcine gut-associated lymphoid tissue, but the presence and function of OFQ receptors in this location have not yet been defined. Loss of the OFQ receptor gene in transgenic mice results in a partial loss of tolerance to opioids, a finding which suggests that OFQ or its receptor may play a role in the development of opioid tolerance (Ueda et al., 1997). It is interesting to note that the constipating actions of opioids do not undergo appreciable tolerance, and in fact represent a clinically undesirable and sometimes life-threatening complication of opioid analgesic therapy (Manara and Bianchetti, 1985). If the OFQ system is a critical factor in the development of opioid tolerance, then it is conceivable that the intestinal OFQ system is fundamentally different from other OFQ-containing sites of opioid action in its patterns of receptor and ligand expression, pharmacological characteristics and ultimate functional role.

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