YFa and analogs: Investigation of opioid receptors in smooth muscle contraction

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Abstract

AIM: To study the pharmacological profile and inhibition of smooth muscle contraction by YFa and its analogs in conjunction with their receptor selectivity.

METHODS: The effects of YFa and its analogs (D-Ala2) YFa, Y (D-Ala2) GFMKFKFMRF amide and Des-Phe-YGGFMKFKFMRF amide in guinea pig ileum (GPI) and mouse vas deferens (MVD) motility were studied using an isolated tissue organ bath system, and morphine and DynA (1-13) served as controls. Acetylcholine was used for muscle stimulation. The observations were validated by specific antagonist pretreatment experiments using naloxonazine, naltrindole and norbinaltorphimine norBNI.

RESULTS: YFa did not demonstrate significant inhibition of GPI muscle contraction as compared with morphine (15% vs 62%, P = 0.0002), but moderate inhibition of MVD muscle contraction, indicating the role of κ opioid receptors in the contraction. A moderate inhibition of GPI muscles by (Des-Phe) YFa revealed the role of anti-opiate receptors in the smooth muscle contraction. (D-Ala-2) YFa showed significant inhibition of smooth muscle contraction, indicating the involvement of mainly δ receptors in MVD contraction. These results were supported by specific antagonist pretreatment assays.

CONCLUSION: YFa revealed its side-effect-free analgesic properties with regard to arrest of gastrointestinal transit. The study provides evidence for the involvement of κ and anti-opioid receptors in smooth muscle contraction.

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Key words: Opioid receptor; Guinea pig ileum; Mouse vas deferens; Smooth muscle contraction; Gastrointestinal motility

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INTRODUCTION

Centrally acting µ-opioid agonists are still the most widely used analgesics for the relief of severe pain, but their...
utility is limited by a number of well-known side effects, including tolerance, physical dependence, respiratory depression, and adverse gastrointestinal effects. To rectify these complications, the effects of opioid drugs on gastrointestinal transit have been extensively studied using rat models. Transit arrest is a common effect of opioids in mammals but the underlying secretomotor changes appear to vary between species\(^6\). Studies on gut muscle contractility have demonstrated that the circular muscle contractility plays a dominant role in segmentation and peristaltic propulsion of the gut\(^2\). Thus, the drug sensitivity of circular muscle contraction differs from that of longitudinal muscle contraction\(^4,6\).

The efforts to study opioids and opioid-receptor pharmacology have largely relied upon the availability of highly subtype-selective agonists and antagonists. Although immunohistochemical studies have revealed that the opioid receptor subtypes, \(\mu\), \(\delta\) and \(\kappa\), are present in the neural tissue of the rat enteric nervous system (ENS), but not in smooth muscle cells\(^8,9\), various other studies have indicated their involvement in intestinal smooth muscle movement. In vitro studies using the charcoal meal method have indicated that \(\mu\) and \(\delta\) receptor activation causes slow transit in rats, but \(\kappa\) receptor activation has negligible effect\(^\text{[10]}\). On the contrary, Mitolo-Chieppa et al\(^\text{[11]}\) have reported the involvement of \(\kappa\)-opioid receptors in inhibiting gut motility. An in vitro study has indicated that activation of both \(\mu\) and \(\delta\) receptors has an inhibitory influence on the peristaltic reflex of the rat ileum\(^\text{[12]}\). Similarly, in vitro studies using electrical stimulation have indicated an inhibitory influence of \(\delta\) receptors (but not of \(\mu\) receptors) on longitudinal muscle contractions in the rat jejunum\(^\text{[13,14]}\). Thus, the ambiguity regarding the role of \(\kappa\)-opioid receptors in gastrointestinal and vas deferens motility still persists. Keeping in mind these findings and current efforts to develop peripherally acting opioid analogues directed towards different opioid receptor profiles (e.g., \(\delta\) agonists or mixed \(\mu\) agonist/\(\delta\) antagonists)\(^\text{[15,16]}\), we designed the methionine-enkephalin-Arg6-Phe7 (MERF)-based chimeric opioid peptide analogs, which have an affinity for multiple opioid receptors, to study tolerance behavior and other side effects of opioids.

MERF peptide has overlapping sequences of Met-enkephalin and FMRF amide, belongs to the opioid family\(^{[16]}\), and is comprehensively distributed in the central nervous system of different mammals\(^{[17]}\). Conversely, peptides of the NPFF [Neuropeptide FF (FLFQPQR-Fa)/FMRFa family antagonize morphine-induced supraspinal analgesia\(^{[18]}\) and may function as endogenous anti-opioid agents\(^{[19]}\). NPFF has also been perceived to exhibit opioid effects along with a role in tolerance. The intriguing relationship between opioid and anti-opioid activity of the peptide can be attributed to the FMRF amino acid sequence at the C terminus of MERF. Along these lines, a chimeric peptide YFa (YGGFMKMKFMRF amide) of met-enkephalin and FMRFa was designed to determine the role of endogenous amphiactive sequences like MERF in analgesia, and its modulation\(^{[20]}\). YFa administered intraperitoneally induces naloxone-reversible antinociception, suggesting the involvement of opioid receptors in mediation of its antinociceptive effects. Moreover, YFa-potentiated morphine induced antinociception and attenuated the development of tolerance to morphine analgesia, suggesting its possible role in pain modulation\(^{[21]}\). mRNA expression studies have revealed that YFa produces \(\kappa\) receptor specific antinociception without any tolerance\(^{[22]}\), and it further induced cross tolerance to 20 mg/kg morphine analgesia after 4 d pretreatment with 80 mg/kg YFa\(^{[23]}\). The results of these studies have been substantiated by forskolin-stimulated cAMP inhibition and Eu-GTP-\(\gamma\)S binding studies\(^{[24]}\).

In addition to YFa, its analogs (D-Ala2) YFa, Y (D-Ala2) GFMMKMKFMRF amide, and Des-Phe (YGGFMKMKFMRF amide) have also been studied. (D-Ala2) YFa (1 mg/mouse) administered intracerebroventricularly (ivc) with 5.86 nmol/L morphine (2 mg/mouse, ivc) produced an additive antinociceptive effect, suggesting its modulatory role in opioid (morphine) analgesia\(^{[21]}\). Furthermore, mRNA studies have indicated that (D-Ala2) YFa acts mainly through \(\delta\) receptors and partially through \(\kappa\) and \(\mu\) opioid receptors\(^{[25]}\), suggesting that D-Ala2 substitution in YFa leads to changes in its receptor selectivity from \(\kappa\) to \(\delta\) subtype. Des-Phe (YGGFMKMKFMRF amide) demonstrates the loss of mRNA expression of \(\mu\) opioid receptor and shows \(\kappa\) opioid receptor agonist activity at a higher concentration (unpublished observations). Thus, the observed tolerance-free antinociception of YFa and its analogs prompted us to examine their other pharmacological properties so as to understand the role of opioid receptors in inhibition of gut motility and vas deferens contraction.

In our previous study, we observed early onset of antinociceptive effect (5 min) by chimeric peptide, YFa, which could be a result of direct opioid receptor stimulation and/or due to release of endogenous opioid peptides. In the present study, in vitro guinea pig ileum (GPI) and mouse vas deferens (MVD) assays were performed. These assays provided a more physiologically favorable environment for the ligand-receptor interaction to understand the peripheral action of the peptides, because these peripheral opioid responses are important for some of their therapeutic properties such as analgesia and side effects like constipation. The effect of opioid receptor activation in these isolated organ preparations is to reduce smooth muscle contraction via inhibition of excitatory neurotransmitter release, which is revealed by measuring the inhibitory action on electrically stimulated contraction of the ileal and vas deferens muscles.

**Materials and Methods**

**Peptide synthesis**

Peptides YFa, (D-Ala2) YFa, (Des-Phe) YFa and Dynor-
phin A (Tyr1-Gly2-Gly3-Phe4-Leu5-Arg6-Arg7-Ile8-Arg9-Pro10-Lys11-Leu12-Lys13) [DynA(1–13)], were synthesized by the solid-phase method on an ACT-90 peptide synthesizer (Advanced ChemTech, Louisville, KY, United States) using the standard chemistry of 9-fluorenlymethoxy carbonyl amino acids (Novabiochem, Laufelfingen, Switzerland) and 1-hydroxybenzotriazole/diisopropylcarbodiimide activation method on Rink amide-MBHA and Wang resin. The peptides were purified by RP-C18 column (mBondapak 10 mm, 7.8 mm × 300 mm; Waters, Milford, MA, United States) on semi-preparative reverse-phase HPLC (Waters 600) with a 40-min linear gradient from 10% to 90% acetonitrile (containing 0.05% trifluoroacetic acid) in water. The mass analysis of the peptides was done in linear positive ion mode by MALDI-TOF/TOF (Bruker Daltonics Flex Analysis, Germany) with 2, 5-dihydroxybenzoic acid as the matrix. The peptide sequence was confirmed by automated peptide sequencing (Proci5e 491; Applied Biosystems, Carlsbad, CA, United States).

### Chemicals

All the chemicals including naloxonazine, naltrindole, norBNI and acetylcholine were purchased from Sigma (St. Louis, MO, United States). Morphine was obtained from AIIMS (New Delhi, India). All the peptides were dissolved in Mili-Q water.

### Animals

Male guinea pigs, 300–400 g (AIIMS), were housed, two per cage, kept on a 12-h light/dark cycle, and fed standard rat chow and water ad libitum. Male albino mice were obtained from Maulana Azad Medical College (Delhi, India). Animals were housed in temperature-controlled room (25 °C ± 1 °C) and exposed to a 12-h light/dark cycle. The animals were handled according to the guidelines of The Committee for the Purpose of Control and Supervision of Experiments on Animals, India, and the Animal Ethical Committee of the Institute of Genomics and Integrative Biology (Delhi, India).

### Bioassay

The experimental procedures were essentially those used previously. For GPI and MVD bioassay, tissue strips were obtained from adult male guinea pigs weighing 300–400 g and male Swiss albino mice weighing 25–30 g. All the animals were sacrificed by intraperitoneal administration of overdose thiopentone (200 mg/kg). Tissues were suspended under 1 g tension in a 10-mL organ bath chamber containing Tyrode solution at 37 °C and bubbled with 95% O₂ and 5% CO₂. The tissues were connected to an isotonic force transducer connected to eight channel organ baths (AD Instruments, Sydney, NSW, Australia) and allowed to equilibrate for 30–45 min. All the tissues were stimulated by chemical method using acetylcholine. Only the tissue preparations that responded to 2 × 10⁻⁶ mol/L acetylcholine by producing contractions of more than 1.5 g tension, were used. Preparations were equilibrated for at least 1 h with washes every 10 min before exposure to drugs. At the start of each experiment, a maximum response to acetylcholine (10⁻⁶ mol/L) was obtained in each tissue to check its suitability and the responses to opioid antagonists were expressed as percentages of the maximum acetylcholine. Each experiment was repeated with at least four separate tissue preparations obtained from different animals. Naloxonazine and naltrindole, specific antagonists of µ and δ opioid receptors, were used as negative controls.

### Data analysis

GPI and MVD muscle contraction was measured as tension in grams. The inhibition percentage was calculated by taking acetylcholine contraction as 100% in all the tissues. All the assays were performed in triplicate and data were analyzed by Student’s t test and one-way ANOVA in ORIGIN version 7.1. The data of each ligand were compared with morphine and DynA(1-13) separately, and P < 0.05 was considered statistically significant.

### RESULTS

#### Effect of YFa on guinea pig ileum muscle contraction

In THE GPI assay (Figure 1), YFa demonstrated negligible inhibition of ileal muscle contraction, even at the highest concentration. Morphine, which interacts through µ opioid receptors, exhibited a highly significant inhibition rate of 62% (P = 0.001) at 10⁻⁶ mol/L and 101% at 2 × 10⁻⁵ mol/L. However, DynA (1-13), a known κ receptor agonist, showed a moderate inhibition of 57% (P = 0.004) at the highest dose of 2 × 10⁻⁵ mol/L.

#### Effect of YFa on mouse vas deferens muscle contraction

In MVD preparations (Figure 2), YFa exhibited a moderate inhibition of 24% (P = 0.001) at 2 × 10⁻⁷ mol/L and 45% at 10⁻⁶ mol/L. The maximum inhibitory response rate was 68% (P = 0.001), which was significantly lower.
than that of morphine (101%) but higher than DynA (1-13) (47%, \( P = 0.004 \)). IC\(_{50}\) of YFa (7.10 \( \mu \text{mol/L} \), \( P = 0.001 \)) was nearly half that of morphine (13.41 \( \mu \text{mol/L} \), \( P = 0.001 \)), (Table 1). Vas deferens preparations pretreated with \( \kappa \) receptor specific antagonist norBNI showed a 44% reversibility of inhibitory activity, whereas, with naltrindole, the \( \delta \) receptor specific antagonist, the activity was declined by only 20%.

**Effect of (D-Ala2) YFa on guinea pig ileum muscle contraction**

In contrast to YFa, (D-Ala2) YFa treatment resulted in moderate inhibition of GPI muscle contraction (Figure 3). It showed escalating inhibition in inhibition from a value of 31.27% (\( P = 0.0006 \)) at 2 \( \times 10^{-7} \) mol/L to 61.51% at 2 \( \times 10^{-5} \) mol/L. The IC\(_{50}\) of (D-Ala2) YFa was 12 \( \mu \text{mol/L} \) (\( P = 0.001 \)) (Table 1) compared with that of morphine (4.44 \( \mu \text{mol/L} \), \( P = 0.001 \)) (Table 1), which again indicated a moderate interaction with GPI muscle. Antagonist pretreatment of ileal tissue with naloxonazine exhibited a 50% decline in inhibition of muscle contraction, whereas only a 20% reversibility was observed with pretreatment with norBNI.

**Effect of (D-Ala2) YFa on mouse vas deferens muscle contraction**

(D-Ala2) YFa demonstrated a considerable inhibition of MVD muscle contraction (Figure 4), which increased progressively from 11.49% (\( P = 0.0006 \)) at 10\(^{10} \) mol/L to 37.22% at 2 \( \times 10^{-4} \) mol/L (\( P = 0.0006 \)). The maximum inhibitory response of 96% (\( P = 0.0006 \)) at 2 \( \times 10^{-4} \) mol/L was comparable to that of morphine (97%, \( P = 0.0006 \)), but significantly higher than that of DynA (1-13) (46%). (D-Ala2) YFa showed an IC\(_{50}\) of 0.20 \( \mu \text{mol/L} \) (\( P = 0.0006 \)) (Table 1), demonstrating the selective interaction of the peptide with \( \delta \) opioid receptors, which are substantially present in MVD muscles.
Pretreatment with naltrindole or norBNI resulted in a 52% (P = 0.0006) and 30% (P = 0.0006) reversibility of inhibition of MVD muscle contraction. DynA (1-13) also showed a weak inhibition of MVD contraction with a non-significant IC50 value.

**Effect of (Des-Phe) YFa on guinea pig ileum muscle contraction**

Moderately significant inhibition was observed with (Des-Phe) YFa treatment comparable to that of DynA (1-13) (Figure 5). The inhibitory response was stronger than that of YFa but weaker than that of morphine. A two-fold increase in inhibition from 15.54% (P = 0.0002) to 32.49% (P = 0.0002) was noted as concentration increased from 10^{-9} mol/L to 2 \times 10^{-6} mol/L with IC50 at 14.9 \mu mol/L (P = 0.0002) (Table 1). The specific-antagonist-pretreated GPI preparations demonstrated that the reversibility in inhibitory activity of (Des-Phe) YFa was higher with norBNI (50%, P = 0.0002) than with naloxonazine (26%, P = 0.0002).

**Effect of (Des-Phe) YFa on mouse vas deferens muscle contraction**

(Des-Phe) YFa treatment resulted in a weak inhibition of MVD muscle contraction (Figure 6). The inhibitory response was comparable to that of dynorphin with a maximum response of 49.78% (P = 0.001) at 2 \times 10^{-4} mol/L. Pretreatment with norBNI resulted in a 48% (P = 0.001) reversibility of inhibitory activity, while naltrindole pretreatment led to a 28% reversibility (P = 0.001).

**Effect of MERF-COOH on guinea pig ileum muscle contraction**

MERF-COOH, an endogenous opioid receptor agonist, has been reported to bind to all three subtypes of opioid receptors. GPI assay (Figure 7) showed a dose-dependent response that was comparable to that of morphine at all concentrations. Analogous to morphine and MERF-COOH, exhibited a steady rise in inhibition at a dose of 10^{-5} mol/L (58.57%, P = 0.005 and 62.10%, P = 0.001, respectively), and further demonstrated a sudden (almost twofold) elevation in inhibition profile by 95.27% (P = 0.005) at the highest dose. The IC50 value (3.71 \mu mol/L, P = 0.005) (Table 1) was comparable to that of morphine (4.40 \mu mol/L, P = 0.001). Moreover, a similar reversibility (50%) in inhibition profile was noted in the GPI preparations pretreated with norBNI and naloxonazine.

**Effect of MERF-COOH on mouse vas deferens muscle contraction**

In the MVD assay (Figure 8), MERF-COOH demonstrated a significant inhibition of MVD muscle contraction. At 10^{-6} mol/L concentration, it exhibited a comparable inhibition profile to morphine, whereas at higher concentrations, the trend varied. MERF-COOH exhibited an IC50 value of 5.51 \mu mol/L (P = 0.001), which was less than half that of morphine (13.41 \mu mol/L, P = 0.001) (Table 1). Moreover, the peptide showed a 72.57% (P = 0.001) inhibition at the highest dose. The δ- and κ-specific antagonist pretreatment of MVD preparations exhibited a similar degree of reversibility (35%) of inhibitory activity with naltrindole and norBNI, respectively.

**Effect of MERF-NH2 on guinea pig ileum and mouse vas deferens smooth muscle contraction**

Contrary to MERF-COOH, MERF-NH2 treatment resulted in a weak inhibition of GPI (41%, P = 0.001) and MVD (31%, P = 0.0005) muscle contraction at the highest concentration of 10^{-4} mol/L (Figures 9 and 10). The specific-antagonist-pretreated preparations of GPI (naloxonazine and norBNI) and MVD (naltrindole and norBNI) did not show any significant reversibility in inhibition profile.
 DISCUSSION

This study examines the effects of YFa and its analogs on GPI and MVD motility, in conjunction with their receptor selectivity. It is well documented that µ opioid receptors are primarily responsible for constipation, along with inhibition of nitric oxide generation [31]. In the gastrointestinal tract, activation of µ opioid receptors results in the inhibition of gut motility that leads to constipation, whereas similar receptors in the central nervous system mediate the analgesic actions of opioids [32]. The µ-receptor-selective drug, morphine, significantly restricted the smooth muscle contractions in lower intestine, indicating the presence of µ opioid receptors in the ileal muscle. Therefore, by inhibiting gastric flow and reducing propulsive peristalsis of the intestine, morphine decreases the rate of intestinal transit. Reduction in gut secretion and increase in intestinal fluid absorption further contribute to the constipating effect [33].

In 1993, Smith and Leslie [34] reported the δ subtype of opioid receptors as the major form in MVD, with a smaller number of µ receptors. Alternatively, in 1999, Pound [35] reported that morphine induced significant inhibition of MVD muscle contraction, which indicated the presence of separate µ opioid receptors. Furthermore, functional interactions between µ and δ opioid receptors, for several biochemical and pharmacological responses have also been reported by various groups [36-39]. These functional interactions of µ receptors could be rationalized on the basis of their indirect activation by δ receptors [40]. Collectively, these findings reveal that δ opioid receptors are prominent in MVD and there exists some cooperation between µ and δ opioid receptors that supports the hypothesis of synergistic interactions.
between these two receptors.

Although the presence of µ receptors in the gut and MVD is well supported in the literature, the role of κ receptors is still ambiguous. Here, we used YFa and its analogs as probes to unravel these hidden aspects. Our previous studies on YFa have revealed its κ-receptor-selective nature. However, at higher concentrations, it also interacts with μ receptors[22-23]. In the present study, YFa showed a negligible inhibition of GPI contraction. This could be due to non-availability of κ opioid receptors or to the counteracting effect of the anti-opioid side (FMRF-amide) of the peptide, through its interaction with the anti-opiate receptors, by increasing sensitivity to cholinergic stimulation upon acetylcholine release[24-25].

To investigate further the reason behind these observations, an analog of YFa, (Des-Phe) YFa, was designed and studied. Upon modification, (Des-Phe) YFa retained its κ-receptor-selective antinociceptive nature but removal of Phe from the C terminus resulted in loss of RF-amide interaction with anti-opiate receptor, hence nullifying the counteractive effect of anti-opiate moiety (RFa) in YFa. (Des-Phe) YFa exhibited a significant inhibition of GPI muscle contractions, comparable to those of dynorphin. In comparison with YFa, a threefold increase in inhibition was observed as a result of the modification. Therefore, the counteractive effect of the anti-opioid side of YFa could be the reason for the non-significant inhibitory effect of YFa. This observation emphasizes the existence of κ-receptor-mediated contractions, in addition to the known μ receptor involvement in GPI muscle contractions[26-28]. Reversibility of contraction by pretreatment with κ-receptor-specific antagonist nor-BNI confirmed the κ-receptor-mediated interaction in GPI.

To substantiate the role of the anti-opiate moiety in the effect of YFa, analogs of MERF were studied. As mentioned earlier, MERF is a well-documented peptide belonging to the opioid family. Here, we studied the inhibitory profiles of two slight modifications of this peptide, MERF-COOH and MERF-NH₂, the latter of which has a C-terminal RFamide residue that interacts with the anti-opiater receptors. As expected, in the GPI assay, MERF-COOH led to a 100% inhibition and MERF-NH₂ resulted in a negligible response. This complete reversal in properties confirms the role of counteractive effects of anti-opioid moieties in antinociception.

In the MVD assay, YFa demonstrated moderately significant inhibition of vas deferens contractions, in a dose-dependent fashion. This observation firmly suggests the involvement of the κ receptors in the observed effect, which corroborates the earlier reports suggesting the involvement of κ receptors in MVD muscle contraction[29-31,42]. Moreover, the maximum inhibitory response was found to be stronger than that of dynorphin (κ-receptor-specific agonist), suggesting the involvement of other receptors also, which may be due to the saturation of κ opioid receptors. The role of κ receptors was substantiated by antagonist pretreatment studies that showed a higher reversibility of contraction by κ-receptor than δ-receptor antagonist. The role of anti-opiate receptor is still not evident in MVD, therefore, that possibility was not considered in this case.

Recently, we have reported that (D-Ala²) YFa mediates its action primarily through δ opioid receptors and partially through μ and κ opioid receptors[32]. In the present study, (D-Ala²) YFa demonstrated a moderate inhibition of GPI muscle contraction, comparable to that of dynorphin, suggesting the involvement of μ and κ receptors in the observed effect. Specific antagonist pretreatment studies have emphasized the role of μ receptors and naloxonazine (μ-receptor antagonist) pretreatment resulted in a 50% reversibility in inhibition. The IC₅₀ value of (D-Ala²) YFa of 12 μM, which was much lower than that of DynA (1-13) (33.8 μM), substantiated the role of μ receptors in (D-Ala²) YFa-mediated GPI contraction.

Furthermore, in the MVD assay, (D-Ala²) YFa demonstrated a significantly greater inhibition than that of morphine at all concentrations up to 10⁻³ mol/L. As expected, this suggests the involvement of δ and μ receptors in MVD muscle contraction. Pretreatment with naltrindole resulted in an almost 50% reduction in inhibition that suggested the involvement of δ receptors, which was further demonstrated by the IC₅₀ value of (D-Ala²) YFa of 0.2 μmol/L (P = 0.01). However, significant inhibition of MVD muscle contraction by morphine (μ-receptor specific) and (D-Ala²) YFa (δ-receptor specific) further signifies that some cooperation may exist between μ and δ receptors in vas deferens preparations[33,34], or the δ receptors may regulate μ-receptor function via heterodimerization[35]. Further studies on heterodimerization of opioid receptors (μ, δ and κ) are required to elucidate their synergistic behavior and are currently in progress in our laboratory.

In conclusion, YFa and its analogs can be viewed as promising candidates to understand the role of opioid receptors in gastrointestinal transit and MVD motility. Although the precise mechanism by which anti-opiate receptors normalize the effects mediated by opioid receptors in GPI and MVD contraction is currently not clear, we provide convincing evidences that anti-opioid receptors are involved in the phenomenon. We also confirmed the presence of κ receptors in GPI and MVD muscles. Furthermore, the present findings provide a systematic approach to advance the researches on opioids due to the similar nature of opioid receptors in GPI and human intestines.

**COMMENTS**

**Background**

To date, centrally acting μ-receptor-specific agonists are the most widely used analgesics but their relieving effect is accompanied by a number of side effects including tolerance and adverse gastrointestinal effects.

**Research Frontiers**

Opioids mediate their effects through various receptors (μ, κ and δ) present in the central nervous system, but the presence of similar receptors in the en-
neric nervous system leads to disturbances in gastrointestinal transit. Previous studies have reported the presence of μ and δ receptors in the gut and vas deferens, whereas the role of κ receptors is still ambiguous. In this study, the authors demonstrated the role of κ receptors and anti-opioid receptors using methionine-enkephalin-Arg6-Phe7 (MERF) peptide analogs.

Innovations and breakthroughs
The study reported VFa, an analgesic peptide molecule, free of gastrointestinal inhibition effect.

Applications
By understanding the roles of various opioid receptors in gastrointestinal transit, this study will provide a systematic approach to advance the researches on opioids.

Terminology
Guinea pig ileum (GPI) and mouse vas deferens (MVD) assays are the well reported methods for screening the drugs/molecules for smooth muscle contractions.

Peer review
Overall, the present work is a useful study which is potentially helpful for establishing the connection between opioid agents and smooth muscle contraction in humans. One goal is to develop pharmacological means to counteract undesirable effects of chronic administration of opioids in patients.

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