ELSEVIER

European Journal of Pharmacology 454 (2002) 53–58

www.elsevier.com/locate/ejphar

Antagonistic effects of CompB on orphanin FQ-induced colonic contractions in rats

Hitoshi Tada[a,b], Kazuhiko Nakagawa[b], Takehira Yamamura[b], Toku Takahashi[a,*]

[a] *Department of Surgery, Duke University Medical Center, Durham, NC, USA*
[b] *Second Department of Surgery, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Japan*

Received 13 August 2002; received in revised form 13 September 2002; accepted 20 September 2002

Abstract

Orphanin FQ has been shown to stimulate colonic contraction without affecting upper gastrointestinal motility in rats. We studied whether a new putative orphanin FQ receptor antagonist, 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1, 3-dihydro-2H-benzimidazol-2-one (CompB), has antagonistic effects on orphanin FQ-induced colonic contractions in vivo and in vitro in rats. Orphanin FQ-(1–17) and orphanin FQ-(1–13) caused contractions of the circular muscle of the distal colon in a concentration dependent manner (10^{-10}–10^{-6} M) in vitro. CompB (10^{-7}–10^{-6} M) caused a significant inhibition on orphanin FQ-(1–17) and orphanin FQ-(1–13)-induced contractions without affecting the spontaneous contractions. Orphanin FQ-(1–17) also caused contractions of the distal colon in vivo. ED_{50} dose of orphanin FQ-(1–17) (400 pmol/kg)-induced contractions were significantly antagonized by CompB (30–300 nmol/kg) in a dose-dependent manner. In contrast, CompB had no inhibitory effects on dynorphin A-induced contractions in vivo and in vitro. These indicate that CompB is a selective orphanin FQ receptor antagonist of the rat colon.

Keywords: Circular muscle; Colonic transit; Dynorphin A

1. Introduction

In 1995, two independent groups identified a 17-amino acid peptide which, despite a structural resemblance to dynorphin A, does not bind to the classical opiate receptors, but it activates an orphan receptor in a *nmol* concentration (Meunier et al., 1995; Reinscheid et al., 1995). This peptide was named orphanin FQ by Reinscheid et al. (1995) because its sequence begins with phenylalanine (F) and ends with glutamine (Q).

We have previously shown that orphanin FQ preferentially stimulates muscle contraction in the rat colon without affecting motility in the upper gastrointestinal tract in vivo (Takahashi et al., 2000b) and in vitro (Takahashi et al., 2000a; Yazdani et al., 1999). We have also shown that orphanin FQ accelerates colonic transit by promoting migrating colonic contractions in rats. In contrast, dynorphin A evoked simultaneous contractions throughout the entire colon which did not migrate aborally (Takahashi et al., 2000b).

Our in vitro experiments have demonstrated that orphanin FQ (10^{-10}–10^{-6} M) caused colonic contractions in a dose-dependent manner. Suramin (a non-selective P_2 purinoceptor antagonist) and reactive blue 2 (a P_{2Y} purinoceptor antagonist), but not pyridoxalphosphate-6-azophenyl-2′,4′ disulphonic acid (PPADS; a P_{2X} purinoceptor antagonist), abolished orphanin FQ-induced colonic contractions. Therefore, it is suggested that orphanin FQ causes muscle contractions by inhibiting purinergic inhibitory motorneurons in the rat colon (Takahashi et al., 2000a).

The orphanin FQ receptors have been classified to be independent of opioid receptors (Meunier et al., 1995; Reinscheid et al., 1995). $[Phe^1\psi(CH_2–NH)Gly^2]NC(1–13)NH_2$, a pseudopeptide analog of orphanin FQ, has been shown to be a selective antagonist of orphanin FQ receptor in the isolated guinea pig ileum and mouse vas deferens preparations (Guerrini et al., 1998). However, our previous study showed that $[Phe^1\psi(CH_2–NH)Gly^2]NC(1–13)NH_2$ (10^{-10}–10^{-7} M) itself caused significant colonic contractions in a dose-dependent manner and had no inhibitory

* Corresponding author. Surgical Service 112, VA Medical Center, 508 Fulton Street, Durham, NC 27705, USA. Tel.: +1-919-286-0411x7883; fax: +1-919-286-1140.
E-mail address: ttakahas@duke.edu (T. Takahashi).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02474-3

effect on orphanin FQ-induced colonic contractions in vitro (Takahashi et al., 2000a). Similarly, another putative orphanin FQ receptor antagonist, nocistatin (30 nmol/kg) (Okuda-Ashitaka et al., 1998), had no inhibitory effects on orphanin FQ-induced colonic contractions (Takahashi et al., 2000b).

On the other hand, it has recently been demonstrated that 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1, 3-dihydro-2H-benzimidazol-2-one (CompB, old nomenclature; J-113,397) is the first potent nonpeptidyl orphanin FQ receptor antagonist with high selectivity over other opioid receptors (Ozaki et al., 2000a,b). CompB inhibited orphanin FQ binding to Chinese hamster ovary (CHO) cells expressing orphanin FQ receptor in a dose-dependent manner (Ozaki et al., 2000b). This suggests that CompB competes with orphanin FQ for receptor binding.

The purpose of this study is to investigate whether CompB has antagonistic effects on orphanin FQ-induced colonic contractions in vivo and in vitro in rats.

2. Methods

2.1. Materials

Orphanin FQ-(1–17) were obtained from Phoenix Pharmaceuticals (Mountain View, CA). Orphanin FQ-(1–13) were synthesized by the Protein and Carbohydrate Structure Facility, University of Michigan. CompB was a gift from Banyu Pharmaceuticals (Tsukuba, Japan).

2.2. In vitro recording of muscular contraction

The animal experiments were carried out in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All efforts were made to minimize animal suffering and to reduce the number of animals used. Male Sprague–Dawley rats (body wt; 230–250 g) were fasted overnight and euthanized by decapitation under xylazine and ketamine anesthesia [13 and 87 mg (kg body weight)$^{-1}$, respectively]. Circular muscle strips were isolated from the distal colon. As previously described (Takahashi et al., 2000a; Yazdani et al., 1999), muscle strips (10 × 3 mm) were suspended between two platinum electrodes in a 30-ml organ bath filled with Krebs–Henseleit buffer (KHB) containing 0.1% bovine serum albumin. KHB was bubbled with 95% O_2 and 5% CO_2 and kept at 37 °C. Mechanical activity was recorded on a polygraph using isometric transducers. Muscle strips were stretched in 1-mm increments and repeatedly exposed to 10^{-6} M carbachol to determine the tissue length providing the maximum contraction (L_0). Carbachol (10^{-6} M) was added into the organ baths 20-min intervals. Individual concentrations of orphanin FQ (10^{-10}–10^{-6} M) were applied into the organ baths at 30-min intervals. Orphanin FQ-induced contractions were observed for 10 min followed by washing. As previously described (Takahashi et al., 2000a), orphanin FQ-(1–17) (10^{-7} M) caused a maximum contraction of the rat distal colon, which produced 45% of carbachol (10^{-6} M)-induced contractions. The maximum contractions induced by orphanin FQ-(1–17) (10^{-7} M or 10^{-6} M) were expressed as 100% and the dose–response curve of orphanin FQ-(1–17) (10^{-10}–10^{-6} M) was constructed without CompB (control study). To study whether CompB affects the dose–response curve of orphanin FQ-(1–17)-induced contractions, muscle strips were preincubated with CompB for 10 min, followed by the application of orphanin FQ-(1–17) (10^{-10}–10^{-6} M) (antagonist study). Before finishing the experiment, carbachol (10^{-6} M) was applied to check whether the contractile activity of the muscle strips was changed throughout the experiment. Our study showed orphanin FQ-(1–17) (10^{-7} M) and carbachol (10^{-6} M)-induced contraction was not significantly changed throughout the experiment in vitro.

We also studied whether CompB has an antagonistic effect on orphanin FQ-(1–13)-induced contractions. There have been several studies investigating the structural requirements for the binding and receptor activation of orphanin FQ. Calo et al. (1997) demonstrated that orphanin FQ-(1–13) was the smallest fragment capable of maintaining the same efficacy and potency as the full peptide,

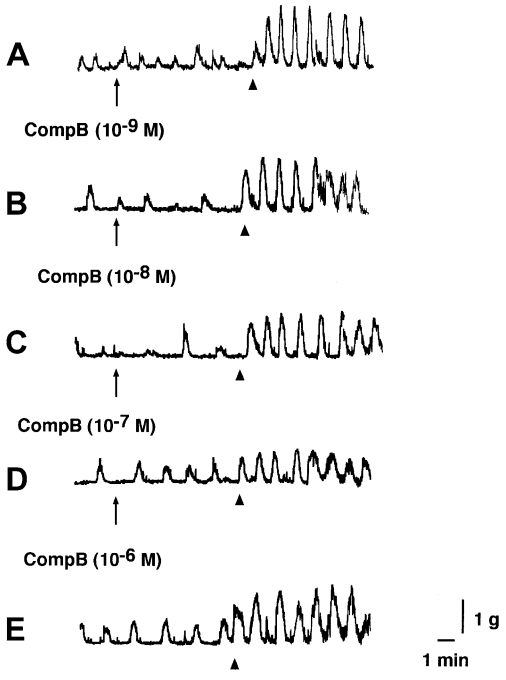

Fig. 1. Effects of CompB (10^{-9}–10^{-6} M) [(A)–(D)] on orphanin FQ-(1–17) (10^{-8} M)-induced contractions of the rat distal colon in vitro. CompB (10^{-9}–10^{-8} M) had no effects orphanin FQ-(1–17)-induced contractions. However, CompB (10^{-7}–10^{-6} M) caused a significant inhibition on orphanin FQ-(1–17)-induced contractions without affecting the spontaneous contractions. After 20 min washing, the inhibitory effects of orphanin FQ-(1–17)-induced contraction was partially recovered (E). Arrow heads indicate the application of orphanin FQ-(1–17) (10^{-8} M). Results were reproducible in five different experiments.

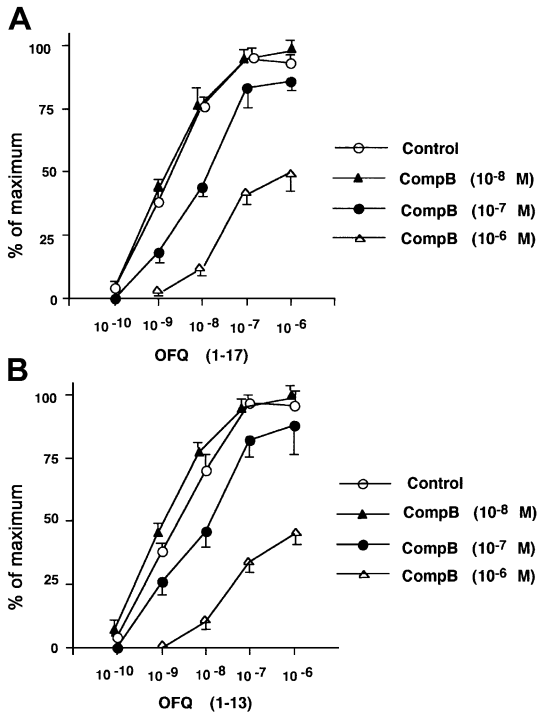

Fig. 2. Effects of CompB (10^{-8}–10^{-6} M) on orphanin FQ-(1–17)- (A) and orphanin FQ-(1–13)- (B) induced contractions of the rat distal colon in vitro. The dose–response curve of orphanin FQ-(1–17) and orphanin FQ-(1–13) (10^{-10}–10^{-6} M) was shifted to the right and downwards by CompB (10^{-7}–10^{-6} M) (Mean ± S.E., $n=5$).

whereas orphanin FQ-(1–12) and orphanin FQ-(1–11) showed a progressive loss of potency in inhibiting electrically stimulated twitch responses of the mouse vas deferens and guinea-pig ileum. Similar observations were made in our previous study of colonic motility. orphanin FQ-(1–13) was as effective as orphanin FQ-(1–17) in stimulating colonic motility, indicating that the entire amino acid sequence may not be necessary for full biological activity (Takahashi et al., 2000b).

2.3. In vivo recording of muscle contraction

Male Sprague–Dawley rats were fasted overnight and anaesthetized with an intramuscular injection of xylazine and ketamine (13 and 87 mg per kg body weight, respectively). To investigate the mode of action of orphanin FQ in the distal colon, an extraluminal force transducer was implanted on the serosal surface of the distal colon to monitor circular muscle contraction, as previously described (Takahashi et al., 2000b). Body temperature was maintained at 37 °C with a heating pad. Bethanechol (40 μg/kg) and ED_{50} dose of orphanin FQ-(1–17) (400 nmol/kg) were administered as a bolus into the jugular vein over a 10-s period. As previously described (Takahashi et al., 2000b), the colonic motor responses to orphanin FQ-(1–17) were reproducible up to five times when orphanin FQ-(1–17) was applied every 20 min. Therefore, orphanin FQ-(1–17) bolus injection was performed every 20 min and evaluated in triplicate and the mean values were calculated.

Orphanin FQ-(1–17) (400 pmol/kg) was administered in the presence and absence of CompB (30–300 nmol/kg). CompB (30–300 nmol/kg) was injected 2 min before orphanin FQ-(1–17) infusion. Before finishing each experiment, bethanechol (40 μg/kg) was administered to check whether the contractile activity was changed throughout the experiment. Our study showed that orphanin FQ-(1–17) (400 nmol/kg)- and bethanechol (40 μg/kg)-induced contractions were not significantly changed throughout the experiment in vivo. At the end of experiment, the rats were euthanized with an intravenous injection of pentobarbital (200 mg/kg).

The area under the curve was calculated using a computer-assisted system (Mac Lab; ADInstruments, Grand Junction, CO) and expressed as a motility index. The area under the curve was evaluated under basal conditions and during orphanin FQ-(1–17) stimulation.

2.4. Statistical analysis

Data were expressed as the Mean ± S.E. Statistical analysis was performed using analysis of variance and paired *t*-test. P values <0.05 were considered as significant.

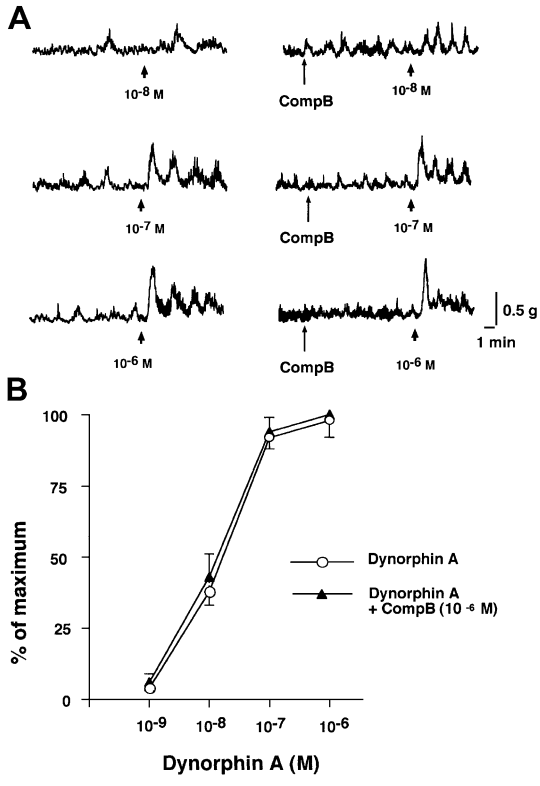

Fig. 3. Effects of CompB (10^{-6} M) on dynorphin A (10^{-8}–10^{-6} M)-induced contractions of the rats distal colon (A) and dose–response curve of dynorphin A (10^{-8}–10^{-6} M) with and without CompB (10^{-6} M) (B) in vitro. Arrow heads indicate the application of dynorphin A (10^{-8}–10^{-6} M). CompB (10^{-6} M) had no inhibitory effects on dynorphin A (10^{-8}–10^{-6} M)-induced contractions of the rat distal colon. Results were reproducible in four different experiments (Mean ± S.E., $n=4$).

3. Results

3.1. In vitro recording of muscular contraction

Orphanin FQ-(1–17) caused contractions of the distal colon in a concentration dependent manner (10^{-10}–10^{-6} M). CompB (10^{-8} M) had no effects on orphanin FQ-(1–17)-induced contractions. However, CompB (10^{-7}–10^{-6} M) caused a significant inhibition on orphanin FQ-(1–17)-induced contractions without affecting the spontaneous contractions (Fig. 1). After 20 min washing of CompB (10^{-6} M), orphanin FQ-(1–17)-induced contraction was partially recovered (Fig. 1). The dose–response curve of orphanin FQ-(1–17) was shifted to the right and downwards by CompB (10^{-7}–10^{-6} M) ($n=5$) (Fig. 2A).

Orphanin FQ-(1–13) caused similar contractions as orphanin FQ-(1–17) in the rat distal colon in vitro. CompB (10^{-7}–10^{-6} M) also caused a significant inhibition on orphanin FQ-(1–13)-induced contractions. The dose–response curve of orphanin FQ-(1–13) was also shifted to the right and downwards by CompB (10^{-7}–10^{-6} M) ($n=5$) (Fig. 2B). In contrast, CompB (10^{-8}–10^{-6} M) had no inhibitory effects on bethanechol (10^{-6} M) (data not shown) or dynorphin A (10^{-8}–10^{-6} M)-induced contractions ($n=4$) (Fig. 3).

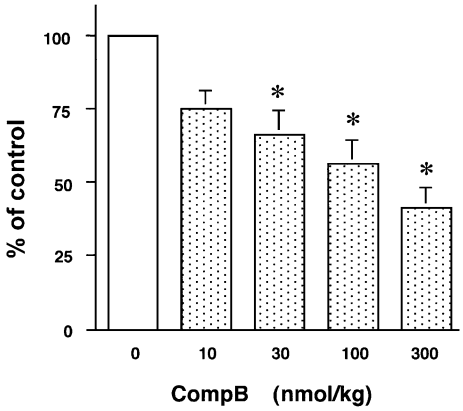

Fig. 5. Effects of CompB (30–300 nmol/kg) on orphanin FQ-(1–17) (400 pmol/kg)-induced contractions of the rat distal colon in vivo. Orphanin FQ-(1–17) (400 pmol/kg)-induced contractions were significantly antagonized by CompB (30–300 nmol/kg) in a dose-dependent manner (Mean ± S.E., $n=5$, $^*P<0.05$).

3.2. In vivo recording of muscle contraction

As previously described (Takahashi et al., 2000b), orphanin FQ-(1–17) caused contractions of the distal colon in a concentration dependent manner (10 pmol/kg–3 nmol/kg). ED_{50} dose of orphanin FQ-(1–17) (400 pmol/kg)-induced contractions were significantly antagonized by CompB

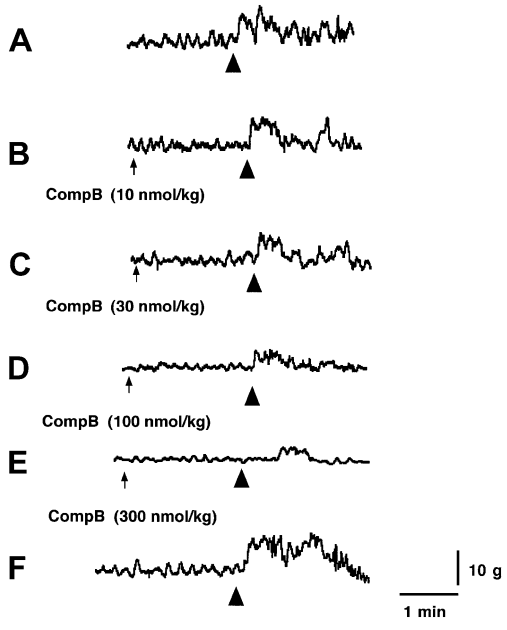

Fig. 4. Effects of CompB (10–300 nmol/kg) ([(A)–(E)] on orphanin FQ-(1–17) (400 pmol/kg)-induced contractions of the rat distal colon in vivo. ED_{50} dose of orphanin FQ-(1–17) (400 pmol/kg)-induced contractions were significantly antagonized by CompB (30–300 nmol/kg) in a dose-dependent manner (10–300 nmol/kg). The inhibitory effect of CompB (300 nmol/kg) disappeared in 30 min (F). Arrow heads indicate the application of orphanin FQ-(1–17) (400 pmol/kg). Results were reproducible in five different experiments.

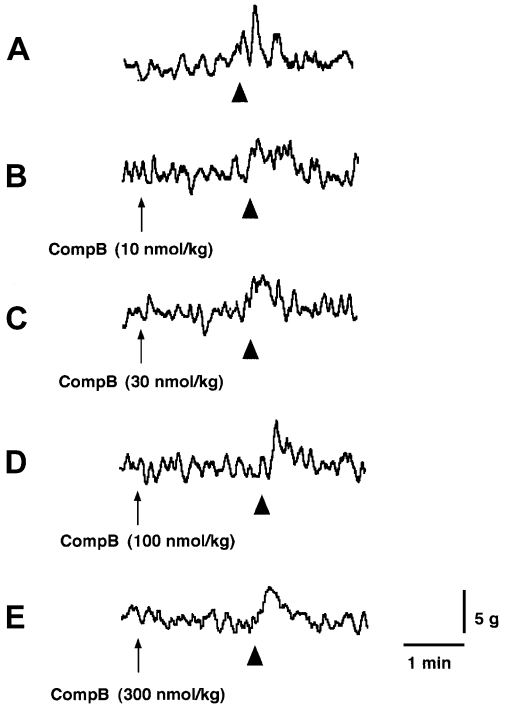

Fig. 6. Effects of CompB (30–300 nmol/kg) on dynorphin A (4 nmol/kg)-induced contractions of the rat distal colon in vivo. CompB (30–300 nmol/kg) had no inhibitory effects on dynorphin A (4 nmol/kg)-induced contractions. Arrow heads indicate the application of dynorphin A (4 nmol/kg). Results were reproducible in four different experiments.

(30–300 nmol/kg) in a dose-dependent manner ($n=5$) (Figs. 4 and 5). The inhibitory effect of CompB (300 nmol/kg) disappeared in 30 min (Fig. 4). In contrast, CompB (30–300 nmol/kg) had no inhibitory effects on ED_{50} dose of dynorphin A (4 nmol/kg)-induced contractions ($n=4$) (Fig. 6).

4. Discussion

We have previously shown that orphanin FQ stimulates muscle contraction in the rat colon without affecting motility in the upper GI tract (Takahashi et al., 2000a,b; Yazdani et al., 1999). Our recent electrophysiological study demonstrated that the inhibitory junction potential in the rat colon is significantly reduced by orphanin FQ and reactive blue 2, a P_2 purinoceptor antagonist (Takahashi et al., 2000a). This suggests that orphanin FQ induces colonic contractions by inhibiting an inhibitory neural pathway (probably an ATP pathway) of the colonic myenteric plexus (Takahashi et al., 2000a).

It is suggested that orphanin FQ-induced contractions are mediated through the intramural myenteric plexus in the rat colon. This possibility is further supported by our in situ hybridization study, in which we demonstrated that orphanin FQ receptors are highly expressed on the myenteric plexus but not on the smooth muscle layers of the rat colon (Takahashi et al., 2000a).

$[Phe^1\psi(CH_2-NH)Gly^2]NC(1-13)NH_2$ has been proposed as a selective antagonist of orphanin FQ receptors in the guinea pig ileum and mouse vas deferens (Guerrini et al., 1998). However, others demonstrated that $[Phe^1\psi(CH_2-NH)Gly^2]NC(1-13)NH_2$ acts as a potent agonist in vivo (Carpenter and Dickenson, 1998) and in vitro (Butour et al., 1998). These differing actions of the pseudopeptide may suggest that different orphanin FQ receptor subtypes are expressed in different tissues.

It has recently been demonstrated that CompB is the first potent nonpeptidyl orphanin FQ receptor antagonist with high selectivity over other opioid receptors (Ozaki et al., 2000a,b). CompB (10^{-9}–10^{-6} M) inhibited orphanin FQ binding to Chinese hamster ovary (CHO) cells expressing orphanin FQ receptor in a dose-dependent manner. In contrast, CompB showed 600-fold or less affinity for mu-, delta- and kappa-opioid receptors. Orphanin FQ-induced suppression of cyclic AMP accumulation elicited by forskolin was completely inhibited by CompB with an IC_{50} value of 26 nM. These results indicate that CompB is a potent and selective antagonist of the orphanin FQ receptor (Ozaki et al., 2000b). When CompB was injected intrathecally or intracerebroventricularly 10 min before the formalin injection, it enhanced the agitation behavior induced by paw formalin injection. This suggested that paw formalin injection induced orphanin FQ release in the spinal cord and the supraspinal brain sites. Endogenously released orphanin FQ produced an analgesic effect and CompB antagonized the analgesic effect of orphanin FQ producing an algesic effect in the rat formalin test (Ichikawa et al., 2001).

There have been several studies investigating the structural requirements for the binding and receptor activation of orphanin FQ. Our recent study of colonic motility has shown that orphanin FQ-(1–13) is as effective as orphanin FQ-(1–17) in stimulating colonic motility, an indication that the entire amino acid sequence may not be necessary for full biological activity (Takahashi et al., 2000b).

In our present in vitro study, CompB (10^{-7}–10^{-6} M) caused a significant inhibition on orphanin FQ-(1–17)- and orphanin FQ-(1–13)-induced contractions without affecting the spontaneous contractions. A similar potency of CompB against orphanin FQ-(1–17) and orphanin FQ-(1–13) would support that the two agonist peptides stimulate the same type of receptors. In contrast, CompB had no inhibitory effects on bethanechol (10^{-6} M) or dynorphin A (10^{-9}–10^{-6} M)-induced contractions. This indicates that CompB is a selective receptor antagonist of orphanin FQ of the rat colon in vitro.

The dose–response curve of orphanin FQ-(1–17) and orphanin FQ-(1–13) was shifted to the right and downwards by CompB (10^{-8}–10^{-6} M). This suggests that CompB has a non-competitive nature in inhibition of orphanin FQ-induced contraction in the rat colon. A classical pharmacological analysis (pA_2 and pK_2) may not be available for orphanin FQ and CompB, because the mechanism of action of orphanin FQ on the colonic contraction seems to be inhibition of a tonic inhibitory input to the colon unmasking contractile activity (Takahashi et al., 2000a). It is noteworthy that much higher concentration of CompB is needed to block the contractile responses to orphanin FQ of the rat distal colon, relative to the values noted in binding studies (Ozaki et al., 2000b). 10^{-9} M of CompB caused a 50% inhibition of orphanin FQ binding (Ozaki et al., 2000b), while 10^{-6} M of CompB had 50% inhibition on orphanin FQ-induced contraction of the rat distal colon in the present study.

We have previously shown that intravenous administration of orphanin FQ (10 pmol/kg–3 nmol/kg) induced contractions of the rat colon in a dose-dependent manner in vivo (Takahashi et al., 2000b). Colonic contractions induced by orphanin FQ were not affected by extrinsic denervation but abolished by tetrodotoxin. Continuous infusion of orphanin FQ and dynorphin A caused similar amount of phasic contractions in the proximal colon. However, the contractile activity induced by the two peptides differed significantly in the mid and distal colon. Giant contractions induced by orphanin FQ infusion migrated from the mid to distal colon. In contrast, dynorphin A evoked simultaneous contractions throughout the entire colon which did not migrate aborally. Subcutaneous administration of orphanin FQ (1–3 nmol/kg) accelerated colonic transit, whereas dynorphin A (30–100 nmol/kg) delayed colonic transit. Therefore, it is suggested that orphanin FQ

accelerates colonic transit by promoting migrating colonic contractions in rats in vivo (Takahashi et al., 2000b).

Our present in vivo study has shown that ED_{50} dose of orphanin FQ-(1–17) (400 pmol/kg)-induced contractions were significantly antagonized by CompB (30–300 nmol/kg) in a dose-dependent manner. In contrast, CompB (30–300 nmol/kg) had no inhibitory effects on dynorphin A (4 nmol/kg)-induced contractions. This suggests that CompB is a selective receptor antagonist of orphanin FQ of the rat colon in vivo as well as in vitro. As CompB has no significant effects of the spontaneous contractions of the rat distal colon, orphanin FQ receptors seem to be not tonically active in the rat distal colon.

Acknowledgements

This study was supported in part by the National Institute of Diabetes and Digestive and Kidney Diseases [(RO1 DK55808 (Toku Takahashi)]. The authors are indebted to Michael Wu and Chris Moore for their technical assistance.

References

Butour, J.L., Moisand, C., Mollereau, C., Meunier, J.C., 1998. [$Phe^1\psi(CH_2-NH)Gly^2$]NC(1–13)NH_2 is an agonist of the nociceptin (ORL1) receptor. Eur. J. Pharmacol. 349, R5–R6.

Calo, G., Rizzi, A., Bodin, M., Neugebauer, W., Salvadori, S., Guerrini, R., Bianchi, C., Regoli, D., 1997. Pharmacological characterization of nociceptin receptor: an in vitro study. Can. J. Physiol. Pharmacol. 75, 713–718.

Carpenter, K.J., Dickenson, A.H., 1998. Evidence that [$Phe^1\psi(CH_2-NH)Gly^2$]NC(1–13)NH_2, a peripheral ORL-1 receptor antagonist, acts as an agonist in the rat spinal cord. Br. J. Pharmacol. 125, 949–951.

Guerrini, R., Calo, G., Rizzi, A., Bigoni, R., Bianchi, C., Salvadori, S., Regoli, D., 1998. A new selective antagonist of the nociceptin receptor. Br. J. Pharmacol. 123, 163–165.

Ichikawa, D., Ozaki, S., Azuma, T., Nambu, H., Kawamoto, H., Iwasawa, Y., Takeshima, H., Ohta, H., 2001. In vitro inhibitory effects of J-113397 on nociceptin/orphanin FQ-stimulated. NeuroReport 12, 1757–1761.

Meunier, J.-C., Mollereau, C., Toll, L., Suaudeau, C., Moisand, C., Alvinerie, P., Butour, J.-L., Guillemot, J.-C., Ferrara, P., Monsarrat, B., Mazarguil, H., Vassart, G., Parmentier, M., Costentin, J., 1995. Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. Nature 377, 532–535.

Okuda-Ashitaka, E., Minami, T., Tachibana, S., Yoshihara, Y., Nishiuchi, Y., Kimura, T., Ito, S., 1998. Nocistatin, a peptide that blocks nociceptin action in pain transmission. Nature 392, 286–289.

Ozaki, S., Kawamoto, H., Itoh, Y., Miyaji, M., Azuma, T., Ichikawa, D., Nambu, H., Iguchi, T., Iwasawa, Y., Ohta, H., 2000a. In vitro and in vivo pharmacological characterization of J-113397, a potent and selective non-peptidyl ORL1 receptor antagonist. Eur. J. Pharmacol. 402, 45–53.

Ozaki, S., Kawamoto, H., Itoh, Y., Miyaji, M., Iwasawa, Y., Ohta, H., 2000b. A potent and highly selective nonpeptidyl nociceptin/orphanin FQ receptor (ORL1) antagonist: J-113397. Eur. J. Pharmacol. 387, R17–R18.

Reinscheid, R.K., Nothacker, H.P., Bourson, A., Ardati, A., Henningsen, R.A., Bunzow, J.R., Grandy, D.K., Langen, H., Monsma Jr., F.J., Civelli, O. 1995. Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. Science 270, 792–794.

Takahashi, T., Bagnol, D., Schneider, D., Mizuta, Y., Ishiguchi, T., Le, P.K., Galligan, J.J., Watson, S.J., Owyang, C., 2000a. Orphanin FQ causes contractions via inhibiting purinergic pathway in the rat colon. Gastroenterology 119, 1054–1063.

Takahashi, T., Mizuta, Y., Owyang, C., 2000b. Orphanin FQ, but not dynorphin A, accelerates colonic transit in rats. Gastroenterology 119, 71–79.

Yazdani, A., Takahashi, T., Bagnol, D., Watson, S.J., Owyang, C., 1999. Functional significance of a newly discovered neuropeptide, orphanin FQ, in rat gastrointestinal motility. Gastroenterology 116, 108–117.