Orally administered soymorphins, soy-derived opioid peptides, suppress feeding and intestinal transit via gut \( \mu_1 \)-receptor coupled to 5-HT\(_{1A} \), D\(_2 \), and GABA\(_B \) systems

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Kaneko K, Iwasaki M, Yoshikawa M, Ohinata K. Orally administered soymorphins, soy-derived opioid peptides, suppress feeding and intestinal transit via gut \( \mu_1 \)-receptor coupled to 5-HT\(_{1A} \), D\(_2 \), and GABA\(_B \) systems. Am J Physiol Gastrointest Liver Physiol 299: G799–G805, 2010. First published July 8, 2010; doi:10.1152/ajpgi.00081.2010.—We previously reported that soymorphins, \( \mu_1 \)-opioid agonist peptides derived from soy \( \beta \)-conglycinin \( \beta \)-subunit, have anxiolytic-like activity. The aim of this study was to investigate the effects of soymorphins on food intake and gut motility, along with their mechanism. We found that soymorphins decreases food intake after oral administration in fasted mice. Orally administered soymorphins suppressed small intestinal transit at lower dose than that of anorexigenic activity. Suppression of food intake and small intestinal transit after oral administration of soymorphins was inhibited by naloxone or naloxonazine, antagonists of \( \mu_1 \)-or \( \mu_2 \)-opioid receptor, respectively, after oral but not intraperitoneal administration. The inhibitory activities of small intestinal transit by soymorphins were also inhibited by WAY100135, raclopride, or saclofen, antagonists for serotonin 5-HT\(_{1A} \), dopamine D\(_2 \), or GABA\(_B \) receptor, respectively. We then examined the order of activation of 5-HT\(_{1A} \), D\(_2 \), and GABA\(_B \) receptors, using their agonists and antagonists. The inhibitory effect of 8-hydroxy-2-dipropylaminotetralin hydrobromide, a 5-HT\(_{1A} \) agonist, after oral administration on small intestinal transit was blocked by raclopride or saclofen. Bromocriptine, a D\(_2 \) agonist-induced small intestinal transit suppression, was inhibited by saclofen, but not by WAY100135. Baclofen, a GABA\(_B \) agonist-induced small intestinal transit suppression, was not blocked by WAY100135 or raclopride. These results suggest that 5-HT\(_{1A} \) activation elicits D\(_2 \) followed by GABA\(_B \) activations in small intestinal motility. We conclude that orally administered soymorphins suppress food intake and small intestinal transit via \( \mu_1 \)-opioid receptor coupled to 5-HT\(_{1A} \), D\(_2 \), and GABA\(_B \) systems.

anorexigenic activity; small intestinal motility; \( \mu_1 \)-opioid receptor; soy \( \beta \)-conglycinin \( \beta \)-subunit

It is known that a number of bioactive peptides derived from enzymatic digest of food proteins exhibit various physiological actions, such as opioid (6, 43, 45), hypotensive (25, 49), cholesterol-lowering (41), anxiolytic (13, 30), and memory-enhancing (32, 42) activities. Among them, several peptides sometimes suppressed food intake after oral administration in rodents (14, 24, 27). We previously reported that hypotensive and vasorelaxing tripeptides, rapakinin (Arg-Ile-Tyr), derived from rapeseed, suppressed food intake via CCK release after oral administration in mice (24). Orally administered tetrapeptide \( \beta \)-lactotensin (His-Ile-Arg-Leu) was derived from bovine \( \beta \)-lactoglobulin through activation of CRF and CGRP receptors (14).

It is generally known that opioid agonists stimulate food intake in animals (5). For example, \( \mu_1 \)-opioid agonists, including morphine or endomorphin-1 or -2, stimulate food intake after central administration in rodents (2, 20); however, we found that soymorphins, \( \mu_1 \)-opioid agonist peptides derived from \( \beta \)-conglycinin \( \beta \)-subunit (soymorphins-5, -6, and -7: Tyr-Pro-Phe-Val-Val, Tyr-Pro-Phe-Val-Val-Asn, and Tyr-Pro-Phe-Val-Asn-Ala, respectively) (30), suppressed food intake after oral administration at a lower dose than that of our previously reported anorexigenic peptides derived from food proteins in mice (14, 24). Since anorexigenic peptides sometimes suppress gastrointestinal motility (24), we then investigated whether soymorphins suppressed gastrointestinal motility. We also examined the mechanism underlying inhibitory activities of soymorphins on food intake and gastrointestinal motility after oral administration using \( \mu \)-receptor antagonists. Gastrointestinal motility is known to be associated with neurotransmitters such as serotonin, dopamine, and \( \gamma \)-aminobutyric acid (GABA). Thus we investigated whether soymorphin-induced small intestinal transit suppression was mediated by the activation of receptors for these neurotransmitters. We also determined the order of activation of these receptors using their agonists and antagonists.

MATERIALS AND METHODS

Materials. Soymorphins were synthesized by the Fmoc strategy. Naloxone, an antagonist of \( \mu \)-opioid receptor, was obtained from MP Biomedicals (Illkirch, France). The \( \mu_1 \)-opioid receptor antagonist naloxonazine dihydrochloride hydrate, the dopamine D\(_2 \) receptor antagonist raclopride, the GABA\(_B \) receptor antagonist saclofen, and the GABA\(_B \) receptor antagonist baclofen were purchased from Sigma-Aldrich (St. Louis, MO). The serotonin 5-HT\(_{1A} \) receptor antagonist WAY100135, the serotonin 5-HT\(_{1A} \) receptor agonist 8-hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT), the dopamine D\(_2 \) receptor agonist bromocriptine mesylate, and the GABA\(_B \) receptor antagonist bicuculline methiodide were from Tocris Cookson (Ellisville, MO). Carboxymethyl cellulose (CMC) was purchased from Wako Pure Chemical Industries (Osaka, Japan).

Animals. Male BALB/c and ddY mice at 7 and 5 wk old, respectively, were obtained from Japan SLC (Shizuoka, Japan). Each mouse was housed under regulated conditions (22°C on a 12-h light-dark cycle with lights on at 7 AM) and had free access to food pellets and water, unless otherwise indicated. All experiments were approved by the Kyoto University animal committee.

Food intake. The food intake experiment was performed as previously described (14, 24, 31, 33). Briefly, individually housed male
BALB/c mice were deprived of food pellets for 18 h with free access to water after acclimation for more than 3 days. Soymorphins (3–39 mg/kg) in saline containing 0.5% CMC were orally (po) injected. Soymorphins (30 mg/kg po) in saline containing 0.5% CMC and the μ-opioid receptor antagonist naloxone (1 mg/kg po) in saline containing 0.5% CMC were coadministered. A combination of soymorphins (30 mg/kg po) in saline containing 0.5% CMC or naloxone (0.3 mg/kg ip) in saline was coadministered. Preweighed food pellets in each cage were measured 20 min and 1, 2, and 4 h after oral administration, and the cumulative food intake was calculated. The food intake experiment started at 11 AM.

**Small intestinal transit.** Small intestinal transit was measured according to previous reports (24, 26). Male ddY mice were also deprived of food pellets for 18 h with free access to water. Peptides with or without antagonists and dissolved in saline were administered orally. Test meal (0.5 ml) [5% Evans blue (wt/vol) suspended in water containing 1% CMC] was orally administered 30 min after oral administration of peptide solution. At 5 min after the test meal administration, the mice were euthanized by cervical dislocation. The abdomen was opened, the small intestine from the pylorus to the ileocecal junction was dissected, and the point to which the test meal had traveled was secured with thread to avoid change in the length of transit due to handling. The distance traveled by the test meal and the total length of the small intestine were measured. Small intestinal transit was calculated as the ratio of the distance traveled by the test meal to the total length of the small intestine and was expressed as a percent. The small intestinal transit experiment started at 11 AM.

**Statistical analysis.** Values are expressed as means ± SE. Statistical comparisons between groups were performed by one-way ANOVA followed by Fisher’s test or the unpaired Student’s t-test. P values < 0.05 were considered significant.

**RESULTS**

**Orally but not intraperitoneally administered soymorphins suppress food intake.** Soymorphin-5 or -7 suppressed food intake at a dose of 30 or 39 mg/kg (equal to 48 μmol/kg), respectively, 2 h after oral administration in mice fasted for 18 h (Fig. 1A). The anorexigenic activity of soymorphin-7 tended to be more potent than that of soymorphin-5. Then we focused on action of soymorphin-7, which suppressed food intake in a dose-dependent manner 2 h after oral administration; the minimum effective dose for the anorexigenic effect was 10 mg/kg (Fig. 1B). Interestingly, intraperitoneally (ip) administered soymorphins did not suppress food intake under our experimental condition (data not shown).

**Orally administered soymorphins suppress food intake via gut μ-receptor.** To investigate whether orally administered soymorphin-7, a μ-opioid peptide, actually suppress food intake through μ-receptor, we used naloxone, an antagonist of μ-receptor. The anorexigenic activities of soymorphin-7 (30 mg/kg po) were blocked by oral administration of naloxone (1 mg/kg), but not by ip administration (0.3 mg/kg) (Fig. 2, A and B). Naloxone alone did not affect food intake at a dose of 1 or 0.3 mg/kg after oral or ip administration, respectively. Taken together, orally administered soymorphin-7 may suppress food intake probably via small intestinal μ-receptor.

**Orally administered soymorphins inhibit small intestinal transit via gut μ1-opioid receptor.** We investigated whether soymorphins affected small intestinal transit. Soymorphin-5, -6, or -7 suppressed small intestinal transit at a dose of 10, 12, or 13 mg/kg (equal to 16 μmol/kg), respectively, after oral administration (Fig. 3A). The rank order of inhibitory activities of soymorphins on small intestinal motility seemed to be consistent with that of anorexigenic activities: soymorphin-7 > -5 > -6. Soymorphin-7 dose dependently suppressed small intestinal transit at a dose of 3–30 mg/kg (Fig. 3B), indicating that soymorphin-7 inhibits small intestinal motility at a lower dose than that necessary for anorexigenic activity. These results suggest that suppression of small intestinal transit by soymorphin-7 may contribute to its anorexigenic activity.

Next, we investigated whether the suppression of food intake and small intestinal motility was based on a similar mechanism using μ-opioid receptor antagonists. The inhibitory effect of soymorphin-7 (10 mg/kg po) on small intestinal transit was also blocked by orally administered naloxone (1 mg/kg), but not by ip administered naloxone (0.3 mg/kg) (Fig. 4, A and B). Orally administered naloxonazine (3 mg/kg), an antagonist selective for μ1-receptor among two subtypes classified pharmacologically, blocked the inhibitory activities of soymorphin-7 (10 mg/kg) on small intestinal transit (Fig. 4C). Nalox-
one or naloxonazine alone did not affect small intestinal motility. These results suggest that the inhibitory effects of soymorphins after oral administration on gastrointestinal transit are mediated by small intestinal μ₁-opioid receptor.

Small intestinal transit suppression of soymorphins is mediated by a novel pathway involving serotonin 5-HT₁A, dopamine D₂, and GABA_B receptors. To investigate whether neurotransmitters, including serotonin, dopamine, or GABA, are involved in the inhibitory activity of soymorphin-7 on small intestinal transit, downstream of μ₁-opioid receptor, we used WAY100135, raclopride, or saclofen, antagonists of serotonin 5-HT₁A, dopamine D₂, or GABA_B receptor, respectively. WAY100135 (10 mg/kg po), raclopride (15 μg/kg po), or saclofen (3 mg/kg po) completely inhibited the soymorphin-7 (10 mg/kg po)-induced delay in small intestinal transit (Fig. 4D). Soymorphin-5-induced suppression of small intestinal transit was also completely blocked by these antagonists (Supplemental Fig. S1; Supplemental Material for this article is available online at the Journal website). Orally administered receptor antagonists alone did not change small intestinal transit (Supplemental Fig. S1). These results suggest that soymorphin suppresses small

Fig. 3. Effect of SMs on small intestinal transit in mice fasted for 18 h. A: SM-5, -6, or -7 inhibited small intestinal transit after oral administration at a dose of 10, 12, or 13 mg/kg, respectively. B: SM-7 dose dependently suppressed small intestinal transit at a dose of 3–30 mg/kg. Each column represents means ± SE (n = 5–6). *P < 0.05, **P < 0.01 compared with each group by ANOVA followed by Fisher’s test; N.S., not significant.

G801 SOYMORPHINS SUPPRESS FOOD INTAKE AND INTESTINAL MOTILITY

AJP-Gastrointest Liver Physiol • VOL 299 • SEPTEMBER 2010 • www.ajpgi.org

Fig. 2. Effect of μ-opioid receptor antagonist on the anorexigenic activity of SM-7. The anorexigenic activity of SM-7 (30 mg/kg po) 120 min after administration was blocked by the μ-opioid receptor antagonist naloxone after oral (po) administration (1 mg/kg, A), but not by intraperitoneal (ip) administration (0.3 mg/kg, B). Each column represents means ± SE (n = 6–8). *P < 0.05, **P < 0.01 compared with each group by ANOVA followed by Fisher’s test; N.S., not significant.

A

SM-7 (p.o.) + Naloxone (p.o.)

Food intake (g/120 min)

0 1.0 1.5

SM-7 Naloxone

− + + −

− − + +

B

SM-7 (p.o.) + Naloxone (i.p.)

Food intake (g/120 min)

0 1.0 1.5

SM-7 Naloxone

− + + −

− − + +

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intestinal transit through activation of 5-HT_{1A}, D_{2}, and GABA_{B} receptors, downstream of μ-opioid receptor.

Order of activation in small intestinal motility is 5-HT_{1A}, D_{2}, and GABA_{B} receptors. We also examined the order of activation of 5-HT_{1A}, D_{2}, and GABA_{B} receptors in small intestinal motility, using their agonists and antagonists. A 5-HT_{1A} receptor agonist, 8-OH-DPAT (10 mg/kg po), delayed small intestinal transit (Fig. 5A). The inhibitory effect of 8-OH-DPAT on small intestinal transit was inhibited by raclopride (15 μg/kg po) or saclofen (3 mg/kg po) (Fig. 5A). The GABA_{B} receptor antagonist baclofen (30 mg/kg po) also decreased small intestinal transit; however, the inhibitory effect of baclofen on small intestinal transit was not blocked by WAY100135 (10 mg/kg po) or raclopride (15 μg/kg po) (Fig. 5C). Taken together, the mechanism underlying the suppression of small intestinal motility may be as follows: serotonin release → 5-HT_{1A} receptor activation → dopamine release → D_{2} receptor activation → GABA release → GABA_{B} receptor activation. Thus we hypothesized that orally administered soymorphins may suppress small intestinal transit via activation of 5-HT_{1A} followed by D_{2} and GABA_{B} receptors, downstream of μ_{1}-opioid receptor (Fig. 5D).

**DISCUSSION**

We found that orally administered soymorphins suppress food intake and small intestinal transit in mice. To the best of our knowledge, soymorphins are the first μ-opioid agonist peptides suppressing food intake after oral administration in mice. The minimum effective dose for inhibitory activity of soymorphin-7 on small intestinal transit after oral administration was approximately one-third of that for anorexigenic activity, suggesting that suppression of small intestinal motility may contribute to its anorexigenic activity. Both the suppression of food intake and gut motility after oral administration of soymorphins was blocked by oral but not ip administered μ-receptor antagonist, supporting our hypothesis that μ-opioid receptor present in the gastrointestinal tract plays an important role in the suppression of food intake and intestinal transit.
role in suppression of food intake and gut motility by soymorphins. We previously demonstrated that soymorphin-5, -6, and -7 inhibits smooth muscle contraction induced by electric stimulation (IC50/μM = 6.0, 9.2, and 13 μM, respectively) in the guinea pig ileum (GPI) assay (30). The rank order of their affinities for the μ-receptor (IC50/μM = 17, 39, and 47 μM, respectively) was consistent with that of the opioid activities on the GPI assay. Soymorphin absorption from the gastrointestinal tract to blood might not necessarily be needed for these activities, because ip-administered soymorphins were inactive, and soymorphin-7, the longest peptide having weakest μ-opioid activity among soymorphin-5, -6, and -7, exhibited the most potent activities.

Central administered soymorphin-5 suppressed food intake at a bolus dose of 30–50 nmol/mouse; however, its anorexigenic activity was not blocked by naloxone (Supplemental Fig. S2), suggesting that the anorexigenic activity after central administration of soymorphin-5 was independent of the μ-opioid system. Suppression of food intake and gut motility after oral administration of soymorphin-5 and -7 was completely blocked by naloxone after oral administration. Thus orally administered soymorphins-induced food intake suppression may be mainly explained by gut motility inhibition via activation of the peripheral μ-opioid system.

Soymorphins suppressed small intestinal motility via μ1-opioid receptor. Morphine, a well-known μ-opioid receptor agonist, suppresses gastrointestinal transit after oral administration, and this suppression was also blocked by oral administration of naloxone (8). Endomorphin-2, a tetrapeptide isolated as an endogenous μ-opioid (48), suppresses gastrointestinal transit via μ1-opioid receptor (46). It was demonstrated that opioid peptides and mRNA of opioid peptide precursors are present in the gastrointestinal tract by immunocytochemistry and in situ hybridization, respectively (1, 9, 18, 22, 34, 35, 47). The μ-opioid receptor is reported to exist in the myenteric and submucosal plexus of the gastrointestinal tract, including
the duodenum or ileum (3, 12, 28, 38), suggesting that μ-opioid system is also present in the gastrointestinal system. It is speculated that orally administered soymorphins might act on the μ-opioid receptor in the smooth muscle associated with gut motility. Furthermore, we confirmed that mRNA of μ-opioid receptor was detected in the mouse small intestine using quantitative real-time polymerase chain reaction (qRT-PCR) (Supplemental Tables S1 and S2).

We demonstrated that the inhibitory effects of soymorphins on gastrointestinal transit were mediated by the activation of 5-HT1A, D2, and GABAB receptors, downstream of the μ1-opioid receptor. We also determined that the order of activation was 5-HT1A, D2, and GABAB receptors.

It was reported that morphine suppressed gastrointestinal transit via serotonin system (36). Serotonin is known to be mainly present in the gastrointestinal tract and released from enterochromaffin cells in the intestine after eating or chemical/toxic stimulation. It was reported that 5-HT1, 5-HT2, 5-HT3, and 5-HT4, which are main subtypes of functional serotonin receptor, are present in the gastrointestinal tract (39). Activation of serotonin 5-HT1A receptor in the enteric nervous system suppresses gut motility (17, 37). It is reported that serotonin 5-HT1 receptor causes a relaxation of the smooth muscle in gastrointestinal tract, whereas 5-HT2, 5-HT3, and 5-HT4 receptors induce a contraction (39). These reports are consistent with our findings that 5-HT1A receptor activation is associated with the soymorphin-induced inhibition of small intestinal transit (Fig. 4D), and 5-HT1A agonist alone suppress it (Fig. 5A). In addition, we detected mRNA expression of 5-HT1A receptor in the small intestine by qRT-PCR (Supplemental Table S2).

It has also been reported that dopamine and a D2 receptor agonist, such as bromocriptine or apomorphine, decreased gastrointestinal transit via D2 receptor, which is present in the small intestine (10, 21). Endomorphin-2 also stimulates dopamine release via μ1-opioid receptor (4). GABA and GABAB agonist also reduced small intestinal motility through the GABAB receptor, which is reported to be located in the small intestine (7, 16, 40). The qRT-PCR experiment revealed that mRNA of dopamine D2 and GABAB receptors was present in the gut (Supplemental Table S2).

Acetylcholine (ACh) release, associated with the acceleration of gastrointestinal motor activity (23), is reported to be inhibited by a μ-opioid receptor agonist, such as morphine or endorphins (29, 44). Serotonin 5-HT1A agonist is also reported to inhibit ACh release (11). Dopamine decreased the release of ACh via D2 receptor (19). GABA and GABAB agonist suppressed ACh release (16). Soymorphins might induce the suppression of gastrointestinal transit through inhibition of ACh release.

Recently, we have found that dipeptide Tyr-Leu (YL) has anxiolytic activity via the activation of serotonin 5-HT1A followed by D1 and GABA receptors (15). It is interesting that common neurotransmitters are involved in the regulation of emotional behavior and gut motility in the central (15) and enteric nervous system (Fig. 5D), respectively, and the order of activations of these neurotransmitters is the same; however, their receptor subtypes were different in the brain and gut system. Because soymorphins have both anxiolytic and gut motility-inhibitory activities after oral administration, further investigation will elucidate whether soymorphins can be applied to prevent the mental stress-induced diarrhea predominant in irritable bowel syndrome.

A number of opioid peptides have been identified from enzymatic digest of food proteins (6, 13, 30, 42, 43, 45). β-Casomorphin, a μ-opioid peptide derived from bovine β-casein, has more potent opioid activity than that of soymorphin in a guinea pig ileum-contraction assay; however, β-casomorphin-5 did not suppress food intake and gut motility after oral administration under our experimental conditions. In contrast, soymorphins suppressed food intake and small intestinal transit. There are a number of splicing variants in μ-opioid receptor. Further investigations will elucidate the interaction of opioid peptides derived from food proteins, including soymorphins, β-casomorphins, and so on, with the splicing variants of μ-receptor. In addition, rubiscolin-6, a δ-opioid agonist peptide derived from α-ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (43), did not suppress small intestinal transit after oral administration (data not shown).

In conclusion, we found that soymorphins suppress food intake and gastrointestinal transit after oral administration. The soymorphin-induced suppression of gastrointestinal transit was mediated by μ1-opioid receptor. The inhibitory effects of soymorphins on gastrointestinal transit also involved serotonin 5-HT1A, dopamine D2, and GABAB receptors, downstream of μ1-opioid receptor.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES
SOYMORPHINS SUPPRESS FOOD INTAKE AND INTESTINAL MOTILITY


