Effects of TAK-637, a Novel Neurokinin-1 Receptor Antagonist, on Colonic Function in Vivo

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ABSTRACT
Substance P (SP) is an important neurotransmitter that mediates various gut functions; however, its precise pathophysiological role remains unclear. In this study, we investigated the effect of SP on colonic function and the effect of TAK-637 {([aR,9R]-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7H-[1,4]diazocino[2,1-g][1,7]napthyridine-6,13-dione}, a new neurokinin-1 (NK1) receptor antagonist, on colonic responses to SP or stress in Mongolian gerbils. SP and the selective NK1 agonist [pGlu6]SP6–11 significantly increased fecal pellet output. TAK-637 reduced [pGlu6]SP6–11-induced defecation, but did not significantly affect neurokinin A-, 5-hydroxytryptamine- or carbachol-stimulated defecation. Oral TAK-637 decreased restraint stress-stimulated fecal pellet output with an ID50 value of 0.33 mg/kg. Ondansetron and atropine, but not the peripheral k-receptor agonist trimetaphine, also reduced restraint stress-stimulated defecation. TAK-637 inhibited the increase in fecal pellet output stimulated by intracerebroventricular injection of corticotropin-releasing factor, but did not affect the stress-induced increase in plasma adrenocorticotropic hormone levels. Denervation of the sensory neurons with capsaicin did not affect stress-stimulated defecation. These results suggest that NK1 receptors in the enteric plexus play an important role in stress-induced changes in colonic function, and that TAK-637 may be useful in the treatment of functional bowel diseases such as irritable bowel syndrome.

SP, NKA, and neurokinin B are members of the tachykinin family of peptides, for which a transmitter status, either in the central or peripheral nervous system, has been established (Otsuka and Yoshioka, 1993). Three main types of receptors, NK1, NK2, and NK3 (which show greater affinity for SP, NKA, and neurokinin B, respectively), mediate the biological actions of the tachykinins. SP is present in the brain, spinal cord, and primary afferent C fibers, and NK1 receptors are involved in nociceptive transmission, emesis, and anxiety (Saria, 1999). SP is also distributed in peripheral tissues such as the intestine and colon, and is especially plentiful in the enteric nervous system. SP exerts a powerful motor-stimulatory action on the intestine and colon by releasing acetylcholine and directly contracting smooth muscles (Koelbel et al., 1989; Scheurer et al., 1994).

IBS is a symptom complex characterized by a chronically abnormal bowel habit (either diarrhea or constipation or both), accompanied in most cases by abdominal pain. IBS is the cause of 20 to 50% of referrals to gastroenterology clinics, and a questionnaire study found it to have a prevalence of 22% (Maxwell et al., 1997). Several pathophysiological factors are involved in IBS, and psychological stress has been reported to be closely associated with an increase in the number of bowel symptoms (Whitehead and Palsson, 1998). Most studies also indicate that visceral hyperalgesia and abnormalities in intestinal and/or colonic motility are the main pathophysiological factors underlying IBS (Jameson and Misiewicz, 1993).

Because the NK1 receptor is involved in gastrointestinal motility and nociceptive transmission, NK1 receptor antagonists are considered to have the potential to ameliorate bowel symptoms in IBS patients. TAK-637 ([aR,9R]-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7H-[1,4]diazocino[2,1-g][1,7]napthyridine-6,13-dione) is a new orally active NK1 receptor antagonist that shows a high affinity (IC50 = 0.45 nM) for NK1 receptors in human IM-9 cells, although its affinity for rat NK1 receptors is low (Natsugari et al., 1999). The affinity of TAK-637 for human NK2 and NK3 receptors is at least 2000 times less than that for NK1 receptors. In the present study, we used Mongolian gerbils (which possess high-affinity NK1 receptors for TAK-637) to investigate the effect of TAK-637 on stress-induced defecation, to clarify whether substance P is involved in stress-induced bowel dysfunction. We also determined the site at which TAK-637 acts to inhibit stress-induced defecation.

Materials and Methods
Animals. Male 17- to 25-week-old MGS/Sea Mongolian gerbils (Seac Yoshitomi, Japan) weighing 60 to 120 g were used in the study.

ABBREVIATIONS: SP, substance P; NKA, neurokinin A; NKB, neurokinin B; NK1, neurokinin-1; IBS, irritable bowel syndrome; CRF, corticotropin-releasing factor; 5-HT, 5-hydroxytryptamine; ACTH, adrenocorticotropic hormone.
Before starting the experiments, the animals were housed under standard controlled environmental conditions, with a 12-h light/dark cycle and food and water provided ad libitum. The care and use of animals and experimental protocol for this study were approved by the Experimental Animal Care and Use Committee of Takeda Chemical Industries Ltd. (Osaka, Japan).

**Drugs and Chemicals.** TAK-637 was synthesized at Takeda Chemical Industries Ltd. Ondansetron was synthesized at Junsei Chemical Co. (Osaka, Japan). Atropine sulfate, salbutamol, capsaicin, Tween 80, and ethanol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Trimebutine maleate, [pGlu6]SP6–11, NKA, methylcellulose, and bovine calf serum were purchased from Sigma Chemical Co. (St. Louis, MO). CRF and SP were purchased from the Peptide Institute Inc. (Osaka, Japan). 5-Hydroxytryptamine (5-HT), carbachol, and aminophylline were obtained from ICN Biomedicals Inc. (Aurora, OH), Tokyo Kasei Co. (Tokyo, Japan), and Eizai Pharmaceutical Co. (Tokyo, Japan), respectively.

TAK-637, atropine sulfate, ondansetron, and trimebutine maleate were suspended in 0.5% methylcellulose solution, and administered at a volume of 4 ml/kg. CRF was dissolved in distilled water and administered at a volume of 5 μl per animal. Aminophylline, salbutamol, and carbachol were dissolved in saline. The capsaicin for sensory nerve deafferentation was dissolved in a solution containing 10% Tween 80, 10% ethanol, and 80% saline (v/v), while the capsaicin for the wiping test was suspended in 0.5% methylcellulose solution. [pGlu6]SP6–11 and NKA were dissolved in dimethyl sulfoxide then diluted in saline containing 0.1% bovine calf serum.

**Normal Fecal Pellet Output.** The day before the experiments, the animals were transferred to individual cages and housed for more than 20 h to allow them to become acclimated to the environmental conditions. The animals were given p.o. vehicle or TAK-637, and the fecal outputs were collected 4 h later.

**SP-Induced Fecal Pellet Output.** Initial experiments to compare the effects of SP and the selective NK1 agonist [pGlu6]SP6–11, on defecation with those of the established stimulant 5-HT were conducted in fed gerbils. The animals were housed individually in grid-floored cages and acclimated to the environment for at least 3 h before starting the experiment. The animals received i.p. SP, [pGlu6]SP6–11, or 5-HT, and the fecal pellets were collected 1 h after the injection.

In the second series of experiments, TAK-637 was administered p.o. and the animals were given i.p. [pGlu6]SP6–11 (1 μg/kg), NKA (10 μg/kg), 5-HT (1 mg/kg), or s.c. carbachol (0.2 mg/kg), 1 h later. The fecal output was measured 1 h after stimulation.

**Restraint Stress-Induced Fecal Pellet Output.** On the day of the experiment, the animals were housed individually for more than 3 h to allow them to become acclimated to the environmental conditions. Each animal in the experimental group was placed into a plastic cylinder, in which it could not turn around. In the unrestrained group, the animals were left in their individual cages without any restraint. In the first series of experiments, we examined the time course of changes in fecal pellet output during a 5-h period of restraint stress. Fecal pellets were collected from each animal every 1 h. In the second series of experiments, which examined the effects of the test drugs, the animals received the test drugs p.o. 1 h before the 2-h restraint stress period. Control animals were given the same volume of vehicle.

**Measurement of Plasma ACTH Concentrations.** Changes in plasma ACTH concentrations during the restraint stress period were measured by radioimmunoassay. The animals were sacrificed by decapitation and their blood was collected just before and 0.25, 0.5, 1, and 2 h after placing them into the cylinders. To prevent coagulation, 6% EDTA solution was added to each blood sample (1/50 by volume) then the blood samples were centrifuged for 15 min at 3000 rpm. The plasma was collected and frozen at −80°C for assay at a later time. The ACTH concentration was measured using a radioimmunoassay kit (ACTH IRMA kit; Mitsubishi Chemical Industries, Tokyo, Japan). In the experiment to examine the effect of TAK-637 on plasma ACTH levels during restraint stress, the animals were given vehicle or 1 mg/kg TAK-637 p.o. 1 h before starting the stress. The animals were killed, and blood samples were obtained 1 h after starting the restraint stress.

**CRF-Induced Fecal Pellet Output.** At 13 or 14 weeks of age, each animal was filmed with a guide cannula for i.c.v. injections of CRF. The animal was placed in a stereotaxic apparatus and anesthetized with pentobarbital sodium (40 mg/kg i.p.) then the scalp musculature was removed to expose the skull. After a hole had been drilled through the skull with a hand-operated drill, the i.c.v. cannula was inserted perpendicularly into the right lateral ventricle (coordinates: 0.6 mm caudal to the bregma, 1.2 mm lateral from the midline, 2.0 mm ventral from the dura) and fixed to the skull with resin. The subsequent experiments were performed at least 2 weeks after surgery. CRF was administered in an injection volume of 5 μl under light ether anesthesia. After the animals had recovered from the anesthetic, they were kept in a quiet room for 2 h and the fecal pellet output was determined. In the experiment to examine the effect of TAK-637 on CRF-induced fecal pellet output, TAK-637 (1 mg/kg) or vehicle was given p.o. 1 h before CRF (3 μg/gerbil i.c.v.) administration, and the number of fecal pellets expelled by each animal was measured 2 h after the CRF injection.

**Stress-Induced Fecal Pellet Output in Sensory-Deafferented Gerbils.** Chemical deafferentation of the sensory neurons with capsaicin was performed according to the method of Takeuchi et al. (1992). Six-week-old animals received s.c. capsaicin once daily for three consecutive days (20, 30, and 50 mg/kg, respectively) under light ether anesthesia. To counteract the respiratory impairment associated with capsaicin injection, the animals were pretreated with i.m. salbutamol (0.1 mg/kg) and aminophylline (10 mg/kg) 10 min before and 2 h after the capsaicin injections. To check the effectiveness of the treatment, one drop of a 0.1-mg/ml solution of capsaicin was instilled into one eye of each animal, and the protective wiping movements were counted. The wiping test was performed again to check the persistence of the treatment 1 week before and 2 days after examining the restraint-stress-induced fecal pellet output.

**Statistical Evaluation.** Because there was no significant difference in the mean weights of the fecal pellets excreted under stress and under unrestrained conditions, the pellet count was considered an adequate measure of fecal output for most of the experiments. However, in the experiment to examine the effect of carbachol on defecation, the total weight of the feces was measured because the feces were watery and uniformed.

All data are expressed as the mean ± S.E.M. The statistical significance of differences between groups was determined using Dunnett’s test or Student’s t test. ID50 values were calculated as the dose of test drug needed to cause 50% inhibition of the stress- or stimulant-induced increase in stool number or weight. The mean number of fecal pellets in the unrestrained group was subtracted from that in each stress-stimulated group, and the ID50 value of the drug was calculated using the inhibitory rate for each dosage group.

**Results**

**Effects of SP and 5-HT on Defecation in Mongolian Gerbils.** SP dose dependently increased fecal pellet output (Fig. 1). [pGlu6]SP6–11 also increased fecal output in a dose-dependent manner, and the increase was significant at 0.1, 1, and 10 μg/kg (Fig. 1). 5-HT also increased fecal pellet output (Fig. 1).

**Effect of TAK-637 on Normal Defecation.** To clarify whether TAK-637 affects the spontaneous excretion of fecal pellets, the effect of TAK-637 on 4-h normal defecation was examined. In vehicle-treated animals, the number of fecal pellets excreted during 4 h was 10.5 ± 1.8 (n = 12). The number of fecal pellets was not inhibited by the administration of TAK-637, i.e., 0.1 mg/kg (10.6 ± 2.1, n = 12), 0.3 mg/kg (8.8 ± 1.9, n = 12), and 1 mg/kg (11.2 ± 2.3, n = 12).
Effect on SP-, NKA-, 5-HT-, or Carbachol-Induced Defecation. To confirm the selectivity of TAK-637 in vivo, its effects on [pGlu6]SP6–11-, NKA-, 5-HT-, and carbachol-induced stool excretion were examined. The i.p. administration of [pGlu6]SP6–11 (1 µg/kg) or NKA (10 µg/kg) significantly increased stool excretion by the gerbils. TAK-637 at 0.1 and 0.3 mg/kg p.o. significantly inhibited the [pGlu6]SP6–11-induced increase in fecal pellet output (Fig. 2). TAK-637 at 0.3 mg/kg slightly inhibited the NKA-induced stool excretion, but the inhibitory effect was not significant. TAK-637 (1 mg/kg) did not affect 5-HT- (1 mg/kg i.p.) or carbachol (0.2 mg/kg s.c.)-induced fecal output (data not shown).

Effect on Restraint Stress-Induced Fecal Pellet Output and Plasma ACTH Levels. The effect of restraint stress on fecal pellet output and plasma ACTH concentrations was investigated. Figure 3 shows the time course of the changes in fecal pellet output during the restraint stress period. Restraint stress significantly increased the number of fecal pellets produced during the 1st and 2nd h, but no significant differences were observed thereafter. Therefore, the effect of the test drugs on the stress-induced increase in fecal pellet output was determined during the first 2 h of restraint stress. Restraint stress markedly increased the plasma ACTH concentration (Fig. 4). This increase became significant 15 min after starting restraint stress and persisted for at least 2 h.

Oral TAK-637 dose dependently inhibited the restraint stress-induced increase in stool excretion with an ID50 value of 0.33 mg/kg (Fig. 5). Ondansetron and atropine sulfate also inhibited the restraint stress-induced increase in stool excretion in a dose-dependent manner, with ID50 values of 0.36 and 0.47 mg/kg, respectively (Fig. 5). Trimebutine maleate did not significantly affect fecal pellet output in response to restraint stress (Fig. 5). The effect of TAK-637 on the increase in plasma ACTH concentrations caused by restraint stress was investigated to clarify whether TAK-637 inhibits the stress response itself. TAK-637 at 1 mg/kg p.o. did not affect the increase in plasma ACTH concentrations caused by restraint stress; 1 h after the start of restraint stress, the plasma ACTH concentration in the vehicle-treated group was 1.14 ± 0.06 ng/ml (n = 7), while that in the TAK-637-treated group was 1.12 ± 0.08 ng/ml (n = 7).

Effect on CRF-Induced Increases in Number of Fecal Pellets. The i.c.v. administration of CRF, a putative mediator of stress responses, increased the number of stools excreted in a dose-dependent manner (data not shown), and the increase in the CRF (3 µg/animal)-treated group was significant. TAK-637 at 1 mg/kg p.o. significantly inhibited these CRF-induced increases in fecal pellet output (Fig. 6).

Effect of Sensory Nerve Deafferentation on Restraint Stress-Induced Fecal Pellet Output. To determine whether sensory neurons are involved in stress-induced defecation, the animals were treated with a high dose of capsaicin to deafferentate the sensory nerves. The fecal pellet counts in the control and sensory neuron-denervated group over a 2-h period under unrestrained conditions were 2.8 ± 0.7 (n = 8) and 3.6 ± 0.7 (n = 7), respectively. Restraint stress significantly increased the fecal pellet output in the control and denervated groups to 13.4 ± 1.0 (n = 8) and 14.7 ± 1.5 (n = 7), respectively, and there was no significant difference between the two groups.
Discussion

IBS is a disorder characterized by altered bowel habits and abdominal pain in the absence of demonstrable pathology. In IBS patients, psychological stress is associated with an increased number of bowel symptoms and stress-reduction therapies result in decreased bowel symptoms and abdominal pain (Maxwell et al., 1997). Psychological or physiological stress induces a complex reaction that involves the activation of both hormonal and neuronal systems, and results in gastrointestinal dysfunction in both humans and animals (Narducci et al., 1985; Williams et al., 1989). Several animal models of stress-associated bowel dysfunction, caused by cold, sound, cold-restraint, and wrap-restraint, have been described. Rat restraint stress models have been widely used, because the technique is simple and the stress does not induce gastrointestinal lesions (Miyata et al., 1992). However, because of the affinity of TAK-637 for rat NK1 receptors is low, for the purposes of this study we used gerbils, whose NK1 receptors have a high affinity for TAK-637. Restraint stress markedly increased the fecal pellet output in the gerbils, as has been reported in rats (Miyata et al., 1992). Exposure to various stressors also induces activation of the pituitary-adrenal axis, resulting in the release of ACTH from anterior pituitary cells, and CRF is involved in this process (Tache et al., 1999). Restraint stress markedly increased plasma ACTH levels in the gerbils, and this increase was sustained for at least 2 h. This duration of elevation is longer than that reported in rats (Williams et al., 1989), and this difference seems to be due to the species difference.

In this study, TAK-637 inhibited restraint stress-induced fecal pellet output in a dose-dependent manner, and its potency was almost the same with that of ondansetron and atropine. Cholinergic neurons and 5-HT3 receptors have been reported to be involved in the stress-induced stimulation of colonic motility (Miyata et al., 1992; Monnikes et al., 1992), and this was confirmed in the present study. Trimebutine, which has been reported to inhibit wrap-restraint stress-induced fecal pellet output in rats (Yamamoto et al., 1998), did not inhibit restraint stress-induced fecal pellet output in gerbils.

One of the most important implications of the present study is that NK1 receptors participate in stress-stimulated changes in colonic function, as illustrated by the finding that SP stimulated fecal pellet output at doses much lower than those required for 5-HT. TAK-637 also inhibited the increase in fecal pellet output caused by [pGlu6]SP6–11, and its inhibi-
itory potency against \([\text{pGlu}^6]\text{SP}_{6-11}\)-stimulated defecation was similar to that against restraint stress-induced defecation. TAK-637 did not affect normal defecation, and it did not suppress the increase in fecal pellet output caused by NKA or carbachol, indicating that it does indeed inhibit stress-induced defecation by blocking NK\(_1\) receptors.

Extensive studies of the distribution of NK\(_1\) receptors in the gut have indicated that, although their locations vary in different parts of the gastrointestinal tract as well as among species, NK\(_1\) receptors are localized mainly in the enteric motor neurons and smooth muscle cells in guinea pig and rat colon (Briejer et al., 1993; Sternini et al., 1995). Many cholinergic neurons in the myenteric plexus show SP immunoreactivity, and SP acts both by stimulating cholinergic pathways via the release of acetylcholine from cholinergic nerve endings and by directly causing smooth muscle contraction (Shuttleworth and Keef, 1995). SP has also been implicated in the atropine-resistant ascending excitatory reflex that occurs in response to balloon distension or electrical field stimulation in isolated guinea pig ileum (Maggi et al., 1994) and in the propulsion of pellets through isolated segments of guinea pig colon (Foxx-Orenstein and Grider, 1996). Endogenous tachykinins are known to be involved in mediating atropine-resistant reflex contractions evoked by distension of the rat duodenum or guinea pig colon in vivo (Giuliani et al., 1993, 1996). In addition, Ramirez et al. (1994) reported that SP as well as acetylcholine is involved in the contractile response to 5-HT\(_3\) agonist in guinea pig ileum. Several studies have indicated that stress activates the branches of the vagus nerve (Monnikes et al., 1992), which contain both cholinergic and noncholinergic excitatory neurons, thus stimulating the release of acetylcholine and SP from the nerve endings in the enteric nervous system.

Because CRF is widely distributed within the brain and induces many effects that are generally associated with stress, it has been proposed to be a “master transmitter,” capable of eliciting coordinated endocrine and autonomic events characteristic of the stress response (Dunn and Berbridge, 1990). Williams et al. (1987) suggested that CRF mediates the effect of stress on gastrointestinal motility and transit, and it has been shown to act in the brain to enhance large bowel transit by stimulating parasympathetic fibers (Monnikes et al., 1992). In this study, TAK-637 inhibited CRF-stimulated fecal pellet output. It has been confirmed that TAK-637 does not have CRF receptor antagonistic activity (unpublished data). Because TAK-637 strongly inhibited the action of centrally administered CRF, it seems to act at the step after the release of CRF.

SP-containing neurons in the gut are localized in the myenteric plexus and the submucosal plexus, and also in the unmyelinated afferent fibers that have their cell bodies in the dorsal root ganglia (Bartho and Holzer, 1985). Large doses of capsaicin produce long-term functional or even morphological ablation of thin sensory neurons (Holzer, 1991). In the present study, the afferent sensory neurons of the gerbils were deafferented with 100 mg/kg capsaicin, a dose reported to be effective in rats (Takeuchi et al., 1992). The finding that stress-induced fecal pellet output was not affected by capsaicin treatment suggests that the SP contained in the capsaicin-sensitive sensory neurons is not involved in the stress-induced stimulation of colonic motility. Our results therefore suggest that TAK-637 inhibits stress-induced fecal pellet output by blocking the NK\(_1\) receptors in the myenteric and/or submucosal plexus in the colon.

Abdominal pain is a key symptom in IBS, and visceral hypersensitivity is currently most widely accepted as the mechanism responsible for abdominal pain in functional bowel disorders (Naliboff et al., 1997). Classical IBS treat-
ments, i.e., antispasmodics and prokinetics, can cause unacceptable side effects and are not always effective against abdominal pain. SP can act as a primary afferent neurotransmitter and has been suggested to mediate nociceptive responses (Bueno et al., 1997). Several studies have also indicated that tachykinin receptor antagonists are effective against pain responses to colonic or intestinal distension in animals (Julia et al., 1994; Mclean et al., 1998), and we have previously found that TAK-637 significantly inhibits the viscerosensory response to colorectal distension in rabbits (S. Okano, Y. Ikeura, and N. Inatomi, unpublished data). These findings suggest that TAK-637 may be effective in reducing abdominal pain in patients with functional bowel disorders.

In summary, both restraint stress and exogenous SP administration caused significant alterations in bowel function in gerbils. TAK-637, a potent and selective NK1 receptor antagonist, inhibited restraint stress-induced fecal pellet output, probably by blocking the peripheral NK1 receptors located in the enteric nervous system. Our data suggest that SP is involved in stress-induced alterations in colonic function. Taken together with the finding that TAK-637 inhibits viscerosensory responses to colorectal distension, TAK-637 seems to be a promising agent for the treatment of IBS and other functional bowel disorders.

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References

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