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Pharmacology, Biochemistry and Behavior 74 (2003) 1005–1013
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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# $NK_1$ receptor-mediated mechanisms regulate colonic hypersensitivity in the guinea pig

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Received 22 October 2002; received in revised form 17 January 2003; accepted 23 January 2003

**Abstract**

Neurokinin-1 ($NK_1$) receptors activated by substance P (SP) are involved in the processing of nociceptive information and are a potential target for therapy of visceral pain. We have evaluated the role of $NK_1$ receptors using a selective antagonist of $NK_1$ receptors in two animal models of colorectal hypersensitivity. The behavioral response to colorectal distension was assessed in freely moving guinea pigs by recording visceromotor reflex contractions of the abdominal musculature. Colonic hypersensitivity was induced by intracolonic administration of a chemical irritant (0.6% of acetic acid), or by acute partial restraint stress. Sensitization was characterized by an exaggerated visceromotor response to a low level of colorectal distension (10 mm Hg). In both models of colonic hypersensitivity, oral administration of TAK-637 (0.1–10 mg/kg) normalized visceromotor responses. The intracerebroventricular (10 μg/kg) or intrathecal (10 μg/kg) administration of TAK-637 inhibited colonic hypersensitivity, suggesting an interaction with central $NK_1$ receptors. In contrast, TAK-637 had no effect on visceromotor responses to colorectal distension at 40 mm Hg in guinea pigs with normosensitive (nonsensitized) colons. In conclusion, central $NK_1$ receptors play a significant role in colonic hypersensitivity induced by visceral afferent nerve sensitization from gastrointestinal origin or acute psychosomatic stress, but not in the perception of colorectal distension in animals with normosensitive colons.


*Keywords:* Colonic hypersensitivity; $NK_1$ receptors; TAK-637; Guinea pig

## 1. Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain and discomfort concurrent with abnormal bowel habits. The etiology and pathophysiology of IBS are poorly understood, and the diagnosis is most often based on the process of exclusion since the symptoms are not related to organic abnormalities. However, an important observation is that the symptoms of IBS result, at least in part, from alterations in visceral perception (Mayer and Gebhart, 1994) and are worsened by anxiety or psychological distress (Drossman, 1999). Although there is no conclusive evidence for differences in somatic pain thresholds, in most subjects with IBS, visceral perception is characterized by hyperalgesia and heightened awareness to visceral stimuli, including luminal distension (Ritchie, 1973; Whitehead et al., 1990; Drossman et al., 1997).

There is strong supportive evidence that substance P (SP), a tachykinin with high affinity for the neurokinin-1 ($NK_1$) receptor, plays a key role as a sensory neurotransmitter involved in the perception of pain and the processing of nociceptive information both in the central and peripheral nervous systems. Immunohistochemical studies have indicated the presence of SP in extrinsic and intrinsic visceral sensory fibers with a particular localization in thin C-type sensory neurons that are sensitive to capsaicin (Holzer, 1991). In the central nervous system, $NK_1$ receptors are expressed in high numbers in the hippocampus, striatum, hypothalamus, amygdala, periaqueductal gray, and the reticulopontine nucleus (Maeno et al., 1993; Mantyh et al., 1984; Nakaya et al., 1994). Central SP-containing neurons have been shown to participate in the processing of noxious and

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0091-3057/03/$ – see front matter © 2003 Published by Elsevier Science Inc.
doi:10.1016/S0091-3057(03)00032-7

stressful stimuli, and the $NK_1$ receptor has been implicated in the modulation of stress responses, mood, and anxiety (Smith et al., 1999; Santarelli et al., 2001). At the level of the spinal cord, $NK_1$ receptors, expressed in high numbers in the dorsal horns, are activated during transmission of primary afferent input (Toda and Hayashi, 1993; Todd et al., 2000) and are involved in the induction of mechanical allodynia (Ma and Woolf, 1995). In addition, SP and $NK_1$ receptors are involved in the transmission of sensory and motor autonomic neurons, which maintain the primary peristaltic activity in the gastrointestinal tract (Grider, 1989; Holzer et al., 1998; Maggi and Schwartz, 1997). The role of tachykinins in visceral nociception has been supported by previous studies (Julia and Bueno, 1997; Julia et al., 1994) demonstrating that intraperitoneal injection of SP or selective neurokinin agonists increases the number of abdominal contractions in response to colorectal distension, suggesting that SP is involved in colonic hypersensitivity. Subsequently, using a series of selective tachykinin antagonists, studies have demonstrated that $NK_2$ receptor-mediated mechanisms are also important in the responses induced by luminal distension of either the jejunum or colon made hypersensitive by inflammation or stress in rodent models (McLean et al., 1997, 1998). Finally, a recent study using knockout mice confirmed the results obtained with selective tachykinin receptor inhibitors, showing that the disruption of the $NK_1$ receptor gene is associated with deficits in visceral pain perception and the loss of secondary hyperalgesia (Laird et al., 2000).

The goal of the present study was to investigate the involvement of $NK_1$ receptor-mediated mechanisms in the development of colonic hypersensitivity using a novel non-peptide antagonist. TAK-637 is characterized by high binding affinity for human and guinea pig $NK_1$ receptors and lower affinity for rat and mouse $NK_1$ receptors. For example, the binding affinity of TAK-637 for the human $NK_1$ receptor in IM-9 cells ($IC_{50} = 0.45$ nM) is higher compared to $NK_1$ receptors in the mouse ($IC_{50} = 82$ nM) and rat ($IC_{50} = 123$ nM) brain (Ikeura et al., 1998). Moreover, in vivo studies showed that TAK-637 has a 900-fold greater potency in the guinea pig compared to other rodents (Natsugari et al., 1999). Because of species-dependent differences in the primary sequence of the $NK_1$ receptor (i.e., the guinea pig receptor being homologous to the human receptor and quite different from the rat receptor), the standard model of colonic hypersensitivity, originally developed in rats by Ness and Gebhart (1988), would not produce reliable data for the effect of TAK-637 in humans. Thus, we adapted and validated the technique for recording of visceromotor behavioral responses induced by colorectal distension in the guinea pig. Our experiments were designed to address the involvement of $NK_1$ receptors in visceral hypersensitivity induced by sensitization of the colon with a mild irritant (intraluminal administration of 0.6% acetic acid) or by partial restraint stress, and to assess the possible therapeutic effect of TAK-637.

## 2. Materials and methods

### 2.1. Experimental animals

Adult male Dunkin–Hartley guinea pigs (Harlan–Sprague Dawley, Indianapolis, IN) weighing 500–600 g were housed under controlled conditions: 20 ± 2 °C, 12-h light–dark cycle, and free access to food and water. The animals were allowed 1 week for initial acclimatization to the animal facility. During the second week of acclimatization, the guinea pigs were brought to the laboratory for 2 h each day (10:00–12:00 a.m.), where they were handled by the investigator to adapt to the laboratory conditions. The experimental design and procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and were approved by the V.A. Animal Care and Use Subcommittee (Oklahoma City, OK).

### 2.2. Implantation of intrathecal or intracerebroventricular cannulas

Guinea pigs were fasted for 24 h prior to the surgical procedures, and intrathecal cannulas were implanted at the $L_5$–$S_1$ spinal cord level under isoflurane anesthesia. Briefly, the $L_5$–$L_6$ junction was exposed and punctured with a 20-gauge needle threaded with a 32-gauge catheter containing a 0.003-in. Teflon stylet. Penetration of the dura was confirmed by twitching of the hind legs. The catheter was then inserted 2 cm laterally along the spinal cord and was secured to the surrounding tissue. A loop was made in the catheter and a single suture was used to secure the catheter in place. At that point, 10 cm of PE 10 tubing was attached to the catheter and tunneled subcutaneously along the spinal column to an incision in the skin at the base of the skull. The final volume of the catheter was between 6 and 8 μl. Both incisions were closed and treated with topical antibiotic/analgesic cream. Following surgery, animals were individually housed and allowed to recover. The placement of the catheter was tested at 2–3 days postsurgery via an injection of 20 μl of 2% lidocaine followed by 10 μl of sterile saline. Only animals that showed transient bilateral paralysis following lidocaine treatment were used for intrathecal injection of TAK-637 or its vehicle.

To implant a chronic catheter for intracerebroventricular administration of TAK-637, guinea pigs were anesthesized with a combination of ketamine (80 mg/kg ip) and xylazine (10 mg/kg ip). The skull was positioned in a stereotaxic frame and a cannula (23-gauge stainless steel) was implanted into the lateral brain ventricle using the following coordinates from bregma: anteroposterior, −2.0 mm; lateral, 2.5 mm; ventral, 4.0 mm. The cannula surrounded by bone wax and gel foam was fixed in place with dental acrylic and the animals were allowed 3–4 days to recover

from the operation before the experiments. Following each experiment, the placement of the catheter into the ventricle was verified by injecting pontamine sky blue 6BX solution and by examining the brain.

### 2.3. Visceromotor response to colorectal distension

The visceromotor behavioral response to colorectal distension was measured using a standard technique for recording the number of abdominal contractions by a strain gauge sutured onto the abdominal musculature. The guinea pigs were fasted overnight prior to the experiment. A strain gauge (RB Products, Stillwater, MI) was positioned to follow the direction of the right external oblique muscle and was carefully sutured (seven standard stitches, 3-0 silk) under anesthesia (2–5%). The skin was sutured over the strain gauge and the lead wires were looped around the animal's flank and secured by a single skin suture. During the experiment, the strain gauge was connected via a shielded cable to a chart recorder to monitor the number of abdominal muscle contractions. A 5-cm latex balloon catheter was inserted via the anal canal 11 cm into the colon and secured with a purse string suture 1 cm above the anal opening. The anesthesia and the surgical procedure lasted about 10 min and the guinea pigs were allowed to recover for 30–45 min before initiating the experiment. Visceromotor responses to tonic colorectal distension were recorded in freely moving animals. Two distension paradigms were used in the experiments; that is, the pressure in the colorectal balloon was increased to either 10 or 40 mm Hg and then maintained constant for a period of 10 min. The number of abdominal muscle contractions recorded during the distension was used as a measure of colonic sensation.

### 2.4. Induction of colonic hypersensitivity

Two models of colonic hypersensitivity were employed in the study. The first model used transient colonic irritation with a mild intraluminal irritant and was adapted from a model of acetic acid-induced colonic hypersensitivity in rats described by Langlois et al. (1994) and then modified by Plourde et al. (1997). In our experiments, guinea pigs were fasted for 24 h and a low-concentration acetic acid (0.6%, 1.5 ml) was administered intracolonically via a silastic tube (ID 0.86 mm). The visceromotor response to colorectal distension at 10 mm Hg measured 1 h after acetic acid instillation was enhanced compared to untreated controls. In the second model, colonic hypersensitivity was induced by partial wrap restraint, which is considered a mild form of psychosomatic stress and has been shown to induce visceral hypersensitivity in rats (Williams et al., 1988; Gue et al., 1997). While the guinea pig was sedated, the fore shoulders, upper forelimbs, and thoracic trunk were wrapped with elastic bandage to partially restrict movement and prevent grooming behavior. The bandages were removed after a 2-h restraint, and then the animals were allowed a 30-min recovery before colorectal distension.

### 2.5. Administration of TAK-637

The guinea pigs were treated with TAK-637 or the vehicle given orally or directly into the central nervous system. Increasing concentrations of TAK-637 were administered by oral gavage of 0.1–10 mg/kg TAK-637 in 2 ml of the vehicle (10% methyl cellulose). The guinea pigs were handled by the experimenter and care was taken to insure that TAK-637 or the vehicle was administered carefully into the oral cavity using a plastic syringe and was swallowed by the animal. Following oral administration, a 1-h period was allowed prior to colorectal distension. To investigate central mechanisms of action, TAK-637 (at a single dose of 10 μg/kg) or the vehicle [10% dimethyl sulfoxide (DMSO)] was administered in a volume of 5 μl directly to the spinal cord via intrathecal infusion or to the brain via intracerebroventricular infusion performed 20 min prior to colorectal distension. In separate experiments designed to assess the effect of chronic treatment, a single oral dose of 3 mg/kg TAK-637 was given daily for a period of 1 or 2 weeks. The duration of action of TAK-637 was studied following the effect of oral administration of a single dose of 3 mg/kg TAK-637 for 24 h. In addition, experiments were performed in nonsensitized guinea pigs to study the effect of TAK-637 (3 mg/kg, oral dose) on visceromotor responses to high-level colorectal distension at 40 mm Hg for 10 min. In all experiments, only a single dose of TAK-637 or the vehicle was examined in each experimental animal.

### 2.6. Drugs and chemicals

IsoFlo was purchased from Abbot Laboratories (North Chicago, IL). DMSO and methyl cellulose were supplied by Sigma-Aldrich (Saint Louis, MO). TAK-637 ((a*R*,9*R*)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione) was synthesized by Takada Chemical Industries (Osaka, Japan) and was supplied by TAP Pharmaceutical Products (Lake Forest, IL).

### 2.7. Data analysis

Visceromotor responses were evaluated by the number of abdominal muscle contractions recorded during the 10-min period of colorectal distension. Values are presented as mean ± S.E.M. Pressure-dependent or time-dependent changes in visceromotor responses to colorectal distension were analyzed by linear regression. Statistical significance of the difference between visceromotor responses in control and experimental groups was analyzed by the nonparametric Mann–Whitney test (no assumption for equal variances). Differences were considered significant at $P < .05$.

## 3. Results

### 3.1. Pressure–response relationship and reproducibility of control responses

The first series of control experiments characterized the relationship between colorectal distension pressure and the magnitude of the visceromotor response in the guinea pig. We found that distension of the colorectal balloon to levels ranging from 10 to 40 mm Hg caused a pressure-dependent linear increase in the number of abdominal muscle contractions measured during the 10-min distension period (Fig. 1). The number of abdominal contractions observed at zero pressure (i.e., when the colorectal balloon catheter is inserted but is not distended during a 10-min recording period) was 2.5 ± 0.9 ($n$ = 7). In the guinea pig, a distension at 10 mm Hg did not cause visible signs of discomfort or pain but produced a visceromotor behavioral response (8–10 abdominal contractions) that is comparable to that produced by innocuous distension at 20–30 mm Hg in a rat model (Ness and Gebhart, 1988). Based upon these findings, a distension pressure of 10 mm Hg was considered innocuous in the guinea pig at control conditions. Under the same conditions, colorectal distension performed at a higher pressure of 40 mm Hg caused a significant increase in the number of abdominal contractions compared to innocuous distension.

A second series of reproducibility control experiments was performed to examine the effect of a succession of distensions in the guinea pig. Four consecutive colorectal distensions at 10 mm Hg applied at 1-h intervals did not alter the magnitude of the visceromotor response (Table 1). However, when the recovery period between colorectal distensions was shorter (10–30 min), the number of abdominal contractions was not reproducible but was rather increased. That is, with each consecutive distension, there was a greater visceromotor response suggestive of colorectal sensitization (data not shown).

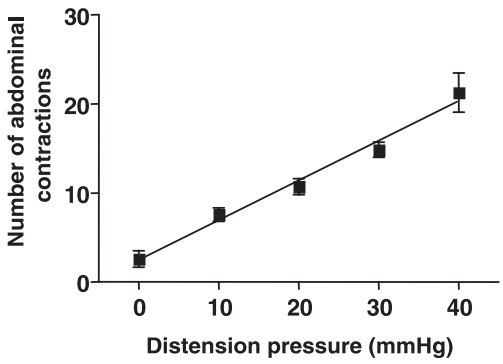


Fig. 1. Pressure–response relationship of the visceromotor responses to colorectal distension in the guinea pig. The number of abdominal contractions recorded during 10-min colorectal distension increases with the increase in pressure. Regression analysis of the responses obtained in three guinea pigs showed linear relationship with a slope of 0.45 ± 0.04 (significantly different from zero, $P$ < .0001).

Table 1
Reproducibility of the visceromotor response to innocuous colorectal distention (10 mm Hg for 10 min) in guinea pigs with nonsensitized colons

| Distension | Number of abdominal contractions |
|---|---|
| Basal | 1.7 ± 0.3 |
| 1 | 7.0 ± 0.6 |
| 2 | 7.0 ± 1.0 |
| 3 | 6.0 ± 1.2 |
| 4 | 6.4 ± 1.9 |

Basal level was measured during a 10-min period before the first distention with the balloon inserted into the colon but not inflated (0 mm Hg). One-hour recovery was allowed between distensions. Values represent the total number of abdominal muscle contractions per 10-min distention at 10 mm Hg expressed as mean ± S.E.M. from three guinea pigs. The responses to Distensions 1–4 fit a straight line with a slope not significantly different from zero ($P$ > .26); that is, there is no increase or decrease of the response due to sensitization or desensitization.

### 3.2. Effects of TAK-637 in models of colonic hypersensitivity

Colonic hypersensitivity was induced by intracolonic administration of 0.6% acetic acid or by partial restraint stress, as described in Materials and methods. The animals with sensitized colons demonstrated a significant increase in the number of abdominal muscle contractions in response to colorectal distension at 10 mm Hg. The exaggerated visceromotor response produced by distension at 10 mm Hg was comparable to that induced by colorectal distension at 40 mm Hg in control animals with normosensitive colons (Fig. 2).

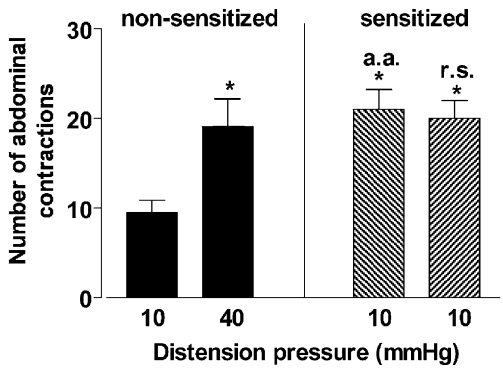


Fig. 2. Models of colonic hypersensitivity. In control guinea pigs with normosensitive (nonsensitized) colons, nociceptive colorectal distension at 40 mm Hg induces a significant increase in the visceromotor response (number of abdominal muscle contractions per 10 min) compared to the response to innocuous colorectal distension at 10 mm Hg. Both intracolonic administration of 0.6% acetic acid (a.a.) 1 h prior to colorectal distension and a 2-h partial wrap restraint stress (r.s.) significantly increased the number of abdominal muscle contractions induced by low-level colorectal distention. Note that the magnitude of visceromotor responses in guinea pigs with sensitized colons is comparable to that induced by a high-pressure colorectal distention (40 mm Hg for 10 min) in normosensitive guinea pigs. Data are the means from four to six animals for each group. * $P$ < .05 compared to responses to colorectal distension at 10 mm Hg in nonsensitized guinea pigs.

In the model of acetic acid-induced colonic sensitization, TAK-637 administered orally at doses of 1, 3, and 10 mg/kg induced a concentration-dependent decrease in the number of abdominal muscle contractions in response to colorectal distension at 10 mm Hg (Fig. 3A). In the model of stress-induced colonic hypersensitivity, TAK-637 induced a concentration-dependent inhibition of the exaggerated visceromotor response to 10 mm Hg at oral doses of 0.3, 1, and 3 mg/kg (Fig. 3B). A comparison between the inhibitory effect of TAK-637 indicated that an oral dose of 3 mg/kg TAK-637 normalized the exaggerated visceromotor response in both models.

### 3.3. Effects of TAK-637 on visceromotor responses in guinea pigs with nonsensitized colon

The experiments were performed to investigate whether TAK-637 has an inhibitory effect on a visceromotor behavioral response produced by a high level of colorectal distension in nonsensitized animals. The visceromotor response induced by colonic distension at 40 mm Hg showed a higher number of abdominal muscle contractions ($21 \pm 2$, $n = 4$) compared to responses to colorectal distension at 10 mm Hg ($8 \pm 1$, $n = 9$; $P < .01$). Oral treatment with 3 mg/kg TAK-637, a dose that significantly inhibited both acetic acid- and stress-induced colonic hypersensitivity, caused no significant change in the number of abdominal muscle contractions ($17 \pm 2$, $n = 5$) induced by colorectal distension at 40 mm Hg in nonsensitized guinea pigs. In control experiments, administration of the vehicle (10% methyl cellulose) had no significant effect on the visceromotor response induced by 40 mm Hg colorectal distension.

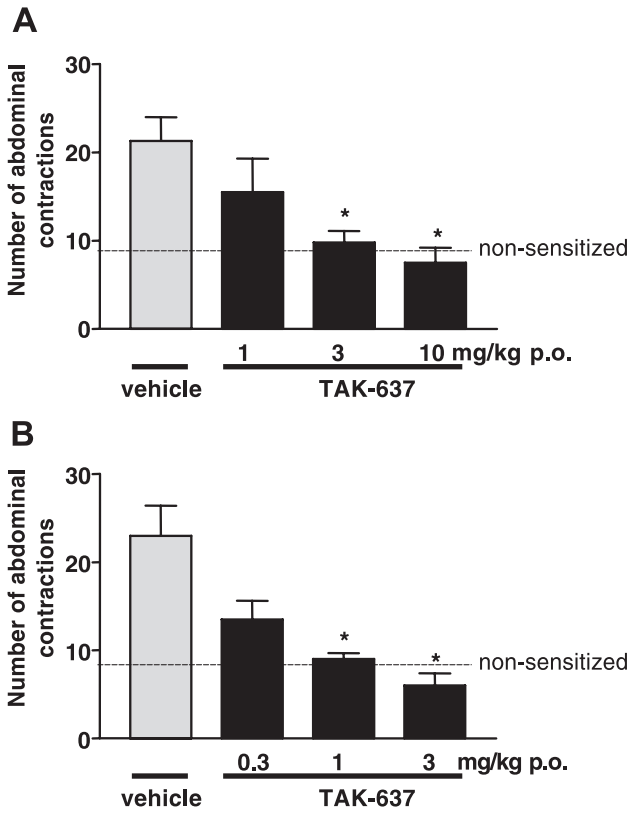


Fig. 3. Effect of TAK-637 on colonic hypersensitivity induced by acetic acid (A) or partial restraint stress (B) in the guinea pig. In both models of colonic sensitization, oral administration of TAK-637 caused a concentration-dependent reduction in the number of abdominal muscle contractions, normalizing the visceromotor response to colorectal distension at 10 mm Hg. Numbers of abdominal contractions per 10-min distention period are expressed as mean ± S.E.M. for four to six guinea pigs for each group. * $P < .05$ compared to responses to colorectal distension in guinea pigs with sensitized colons treated with the vehicle (10% methyl cellulose).

Table 2
Duration of action of TAK-637 (3 mg/kg po) against acetic acid-induced colonic hypersensitivity measured by the visceromotor response to innocuous colorectal distention (10 mm Hg for 10 min)

| Hours predose of TAK-637 | Number of abdominal contractions |
|---|---|
| 0 | 22.3 ± 1.2 |
| 1 | 9.3 ± 1.8 * |
| 3 | 9.0 ± 2.5 * |
| 12 | 6.7 ± 1.5 * |
| 15 | 10.7 ± 0.9 * |
| 24 | 23.7 ± 0.7 |

Colonic hypersensitivity was induced by intracolonic infusion of acetic acid (0.6%) 1 h prior to the measurement of a visceromotor response. Values represent the total number of abdominal contractions per 10-min distention period at 10 mm Hg, expressed as mean ± S.E.M. from three to five guinea pigs for each time point.

* $P < .05$ compared to time point zero (no treatment).

### 3.4. Duration of action of TAK-637 following single oral administration

The duration of action of TAK-637 was established using the model of acetic acid-induced visceral hypersensitivity. The effect of a single oral dose of 3 mg/kg on the number of abdominal contractions evoked by 10 mm Hg distension was studied at 1, 3, 12, 15, and 24 h after the dosing. The data showed that the inhibitory effect of TAK-637 remains statistically significant up to 15 h following treatment (Table 2).

### 3.5. Efficacy of TAK-637 following multiple dosing

The phenomenon of receptor desensitization is associated with the repetitive effects of selective agonists or antagonist on $NK_1$ receptors. The present experiments were designed to determine whether multiple oral treatments with an efficacious dose of TAK-637 result in any changes in efficacy. Guinea pigs received an oral dose of 3 mg/kg TAK-637 given daily at 9 a.m. for 6 days. On the seventh day, colonic hypersensitivity was induced by acetic acid and the visceromotor response to colorectal distension at 10 mm Hg was recorded. The results are presented in Fig. 4 and indicate that following 1 week of daily treatment with TAK-637, there was a significant increase in acetic acid-induced

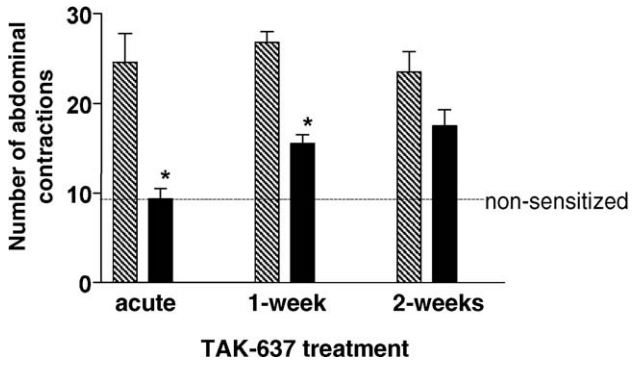


Fig. 4. Effects of continuous oral treatment on the efficacy of TAK-637 in the guinea pig model of acetic acid-induced colonic hypersensitivity. TAK-637 at a dose of 3 mg/kg (filled bars) or the vehicle (10% methyl cellulose; shaded bars) were applied daily during 1 or 2 weeks of treatment.

colonic hypersensitivity. However, when the treatment period was extended to 2 weeks, the efficacy of TAK-637 was not manifested.

### 3.6. Site of action of TAK-637

Oral administration of TAK-637 demonstrated high efficacy in models of colonic hypersensitivity normalizing the visceromotor response to innocuous colorectal distension. The possible central sites of action of TAK-637 were investigated by studying the effect of spinal or supraspinal administration of TAK-637 against colonic hypersensitivity induced by either partial restrain stress or by intracolonic acetic acid. A single dose of 10 μg/kg TAK-637 was applied at a volume of 5 μl given either intrathecally or intracerebroventricularly 20 min prior to recording the response to colorectal distension at 10 mm Hg. Both the spinal and supraspinal applications of TAK-637 completely inhibited colonic hypersensitivity induced by acetic acid (Fig. 5A). Similarly, both intrathecal and intracerebroventricular administrations of TAK-637 resulted in complete inhibition of colonic hypersensitivity induced by partial restraint stress (Fig. 5B). In control experiments, spinal or supraspinal administration of the vehicle (5 μl DMSO) had no effect on the visceromotor response produced by colorectal distension at 10 mm Hg in guinea pigs with colonic sensitization.

## 4. Discussion

Our results demonstrate that $NK_1$ receptor-mediated pathways are involved in colonic hypersensitivity and can be effectively inhibited by oral or direct (intrathecal or intracerebroventricular) administration of TAK-637. Oral dosing of the selective $NK_1$ receptor antagonist, TAK-637, normalized the exaggerated visceromotor response in both models of acute colonic hypersensitivity and the inhibitory effect of TAK-637 was achieved within the first hour and remained significant up to 15 h following treatment. Normalization of the visceromotor response was also obtained following central administration of TAK-637. Thus, it is evident that the pathways involved in visceral hypersensitivity induced by an acute psychosomatic stress paradigm (partial wrap restraint) or by an acute acetic acid-induced sensitization of colonic afferents are sensitive to the blockade of central $NK_1$ receptors. The exact SP-mediated mechanisms of colonic sensitization following acetic acid challenge of the colon or acute restraint stress are unknown and there are multiple possible scenarios that could explain the effects of TAK-637. Although it is well documented that the $NK_1$ antagonist exerts an anti-inflammatory action (Gao, 1999), it is unlikely that the normalization of colonic hypersensitivity in our experiments reflects an effect of TAK-637 on colonic inflammation. This assumption is based on the fact that visceral sensitization induced by intracolonic administration of acetic acid (0.6%) is not associated with colonic inflammation (Langlois et al., 1994; Plourde et al., 1997; Gibson et al., 2001). Moreover, we believe it is more likely that colonic afferents are the

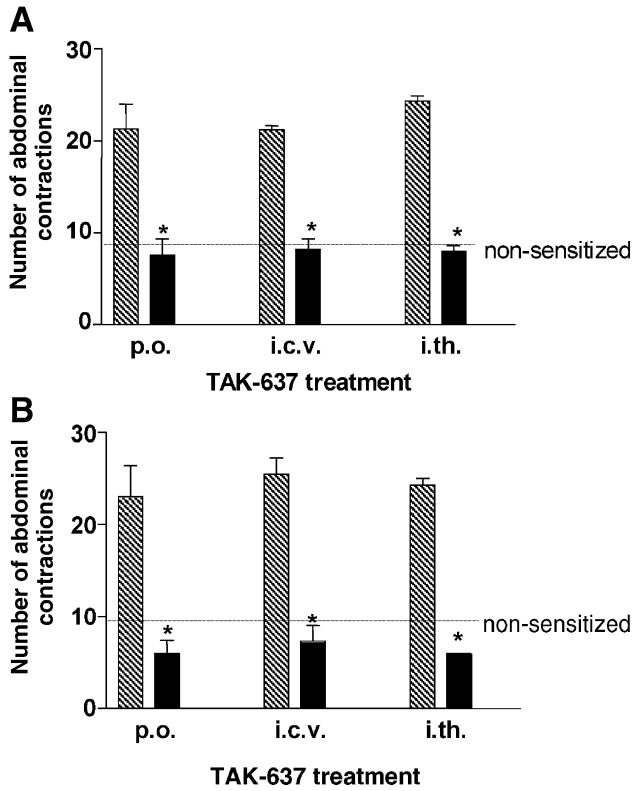


Fig. 5. Comparison between the effects of TAK-637 on the visceromotor response to colorectal distension at 10 mm Hg following three different routes of administration in models of acetic acid-induced (A) or restraint stress-induced (B) colonic hypersensitivity. TAK-637 was given orally at a dose of 10 mg/kg in 10% methyl cellulose or by either intracerebroventricular (icv) or intrathecal (ith) infusion at a dose of 10 μg/kg in DMSO. The effects of TAK-637 (filled bars) or the vehicle (shaded bars) are expressed as mean ± S.E.M. from four to six experiments in each group. * $P < .05$ compared to respective vehicle.

main target of acetic acid-induced sensitization, since it has been reported that rats treated with capsaicin or a CGRP antagonist did not develop colonic hypersensitivity in response to intracolonic infusion of 0.6% acetic acid (Plourde et al., 1997). Consequently, sensitization of neurons within the dorsal horn of the spinal cord that receive and process information from visceral afferents is a key mechanism in the development of visceral hyperalgesia (Gschossmann et al., 2001). Tachykinin receptors at the postsynaptic site could be a major factor in the development of colonic sensitization because electrophysiological recordings from dorsal horn neurons have shown that colonic distension does lead to increased neuronal activity that can be blocked by both $NK_1$ and $NK_2$ receptor antagonists (Bueno et al., 1997; Laird et al., 2001). Furthermore, supraspinal sensitization has been suggested as a key component in the development of stress-induced colonic hypersensitivity. There is strong supportive evidence that the amygdala, an important limbic structure engaged in reflex stress responses to aversive stimuli, modulated the effect of stressful conditions on the development of gastric pathology (Henke et al., 1991). Recently, we showed that direct modulation of the rat amygdala with the glucocorticoid corticosterone induces colonic hypersensitivity (Greenwood-Van Meerveld et al., 2001). Furthermore, postsynaptic $NK_1$ receptors are highly expressed in the amygdala (Maeno et al., 1993) and immobilization stress has been associated with endocytosis of central $NK_1$ receptors (Smith et al., 1999). In a model of stress-related anxiety induced by separation of the pups from their mother, vocalizations have been suppressed by selective inhibitors of central $NK_1$ receptors administered to guinea pigs or by genetic deletion of the $NK_1$ receptor in mice (Rupniak et al., 2000). In our laboratory, we have established similar antianxiety effects of TAK-637 in guinea pig pups (unpublished data). With respect to the role of $NK_1$ receptors in visceral sensitivity, studies using $NK_1$ receptor knockout mice have confirmed the results obtained with selective tachykinin receptor inhibitors, showing that the disruption of the $NK_1$ receptor gene is associated with deficits in visceral pain perception and the loss of secondary hyperalgesia (Laird et al., 2000). Finally, in animal models of psychological stress, stress-induced visceral hypersensitivity involves activation or sensitization of mast cells in the gastrointestinal tract (Gottwald et al., 1995; Gue et al., 1997; Monnikes et al., 2001) as well as activation of macrophage function linked to the expression of proinflammatory cytokines (Bueno and Fioramonti, 1999).

To find the location of the $NK_1$ receptors involved in visceral hypersensitivity, we investigated the effects of TAK-637 following direct infusion via chronically indwelling intrathecal or intracerebroventricular cannulas. The observation that both spinal and intracerebroventricular administrations of TAK-637 inhibit colonic hypersensitivity induced by either acetic acid or partial restraint stress suggests that the inhibitory effects of TAK-637 occur through a central mechanism of action. Our findings are compatible with recent observations in a rabbit model, showing that TAK-637 inhibits viscerosensory responses to colorectal distension interacting mainly with $NK_1$ receptors in the spinal cord (Okano et al., 2002). TAK-637 permeates the blood–brain barrier and may exert its effect through inhibition of central $NK_1$ receptors following oral administration. Within the central nervous system, SP is the most abundant tachykinin and $NK_1$ receptors are expressed at high levels in brain regions involved in the regulation of affective behavior and neurochemical responses to stress in mammalian species including humans. The activation of supraspinal SP pathways in response to noxious or aversive stimulation was first demonstrated in animal models, where changes in SP content in the hippocampus, the septum, and the ventral tegmental area were found after inescapable foot shock, immobilization, and social isolation (Herman and Panksepp, 1978). A recent study examining the distribution of $NK_1$ receptor immunoreactivity in the guinea pig brain discovered that $NK_1$ receptors are present at sites that are comparable to these seen previously in other rodents (Yip and Chahl, 2001). The functional significance of supraspinal $NK_1$ receptors in the regulation of stress-induced behavior is supported by the inhibition of colonic hypersensitivity following intracerebroventricular administration of TAK-637 as well as by a previous observation (unpublished data) showing that TAK-637 is a potent antagonist of the anxiety-like behavior produced in guinea pigs by intracerebroventricular administration of SP. However, the relative contribution of central $NK_1$ receptors expressed in specific spinal cord and brain areas to colonic hypersensitivity remains to be investigated because intracerebroventricular administration still allows small amounts of the compound to circulate through the ventricular cerebrospinal fluid into the spinal region and vice versa.

In previous studies, spinal administration of SP antagonists in rats has been associated with motor dysfunction (Vaught and Scott, 1987; Traub, 1996). The deficit in motor function has not been attributed to a selective blockade of $NK_1$ receptors, but rather to the chemical structure of the compound because equal motor dysfunction was induced by a stereoisomer with no binding affinity for the $NK_1$ receptor (Yamamoto and Yaksh, 1991). In our experiments, a complete inhibition of acetic acid- or partial restraint stress-induced hypersensitivity was found following central administration of 10 μg/kg TAK-637, a relatively low dose, which showed no visible effects on the motor function of freely moving nonanesthesized guinea pigs. Also, there was no visible motor impairment following oral administration of TAK-637 at doses ranging from 0.3 to 10 mg/kg. Our findings are in agreement with the results of a recent study evaluating rotarod performance of rats following treatment with selective nonpeptide tachykinin antagonists, suggesting that spinal $NK_2$ and $NK_3$, but not $NK_1$, receptors are involved in motor control (Kamp et al., 2001).

In guinea pigs with normosensitive colons, we found that TAK-637 had no significant effect on the visceromotor response to colorectal distension at a relatively high distension pressure (40 mm Hg), despite the fact that the response was similar to that obtained by colorectal distension at 10 mm Hg in the guinea pigs with sensitized colon, which was normalized by TAK-637. This intriguing observation indicates that $NK_1$ receptors are not a major factor in the transmission of "normal" colonic sensation but are involved in the process of colonic sensitization. Our finding is supported by previous observations showing that in rats with nonsensitized colon, $NK_2$, but not $NK_1$, receptors mediate the visceromotor response associated with normal pain perception in the rat (Julia and Bueno, 1997; Julia et al., 1994). In a broader sense, the findings support the opinion that the origin of abdominal pain in patients with stress-related functional gastrointestinal disorders is the development of colonic hypersensitivity, when nonpainful stimuli are perceived as painful (allodynia), rather than the development of abnormal motility patterns associated with nociceptive levels of colonic distension. Clinical studies have confirmed that IBS patients have a decreased pain threshold in response to luminal distension compared to healthy subjects (Ritchie, 1973; Whitehead et al., 1990). Moreover, this hypersensitivity can be correlated to an earlier sensitizing event such as an acute enteric infection, a stressful life event, or a history of physical/sexual abuse in childhood (Drossman, 1999).

In conclusion, normalization of the visceromotor response to colorectal distension induced by TAK-637 in two models of colonic hypersensitivity using conscious, freely moving guinea pigs suggests that $NK_1$ receptors are part of the sensory pathways linked to sensitization of the colon by activation of intraluminal receptors by an intraluminal irritant (0.6% acetic acid) or by a mild psychosomatic stress (partial wrap restrained). In contrast, $NK_1$ receptors do not seem to be a major factor in the perception of colorectal distension at relatively high pressure in animals with normosensitive colon. Based on the findings, TAK-637 is a promising candidate for the treatment of abdominal pain in patients with IBS, considering that it is active after oral administration and inhibits colonic hypersensitivity by interacting with central rather than peripheral $NK_1$ receptors. However, our results showed that chronic treatment with TAK-637 results in desensitization and reduction in therapeutic activity, a feature that is common for nonpeptide $NK_1$ receptor antagonists and is most likely due to internalization of $NK_1$ receptors. Despite this observation in guinea pigs treated with TAK-637, clinical studies in patients with colonic hypersensitivity are required to fully examine the effect of chronic therapy with TAK-637 on therapeutic efficacy.

## Acknowledgements

Financial support for this study was provided by TAP Pharmaceutical Products.

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