Peripheral Activity of a New NK₁ Receptor Antagonist TAK-637 in the Gastrointestinal Tract

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ABSTRACT

Pathways controlling gastrointestinal function involve the activation of neurokinin NK₁ receptors by substance P (SP) under normal and pathological conditions. Our aim was to pharmacologically characterize the effect of a nonpeptide NK₁ receptor antagonist TAK-637 [(αR,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]naphthyridine-6,13-dione] and determine key mechanisms of TAK-637 action in the gastrointestinal tract. Experiments were performed using intestinal preparations isolated from the guinea pig. The selective agonists of NK₁ receptors, [Sar⁹,Met(O₂)¹¹]-SP and GR 73632 [H₂N-(CH₂)₄-CO-Phe-Phe-Pro-NMe-Leu-Met-NH₂], induced contractions in colonic longitudinal muscle pretreated with atropine. TAK-637 (1–100 nM) caused a rightward shift of the concentration-response curves showing nanomolar affinity against [Sar⁹,Met(O₂)¹¹]-SP (Kᵢₕ = 4.7 nM) and GR 73632 (Kᵢₕ = 1.8 nM). This antagonist effect remained unchanged by tetrodotoxin. Furthermore, neither the contractions of colonic circular muscle induced by selective activation of NK₂ receptors by GR 64349 (Lys-Asp-Ser-Phe-Val-Gly-R-γ-lactam-Leu-Met-NH₂) nor the responses of taenia coli induced by the selective NK₃ receptor agonist senktide were affected by TAK-637 (100 nM). Studies of electrically induced neurogenic contractions showed that TAK-637 had no effect on cholinergic responses to single-pulse (0.5 ms) stimulation or stimulation with increasing frequency (1–16 Hz, 0.5 ms, 5-s train duration). In contrast, TAK-637 significantly reduced nonadrenergic, noncholinergic contractions of colonic longitudinal muscle evoked at frequencies of 8 to 16 Hz and prevented the development of capsaicin-induced contractions in isolated segments of terminal ileum. Our results indicate that TAK-637 is a selective antagonist of smooth muscle NK₁ receptors that activate intestinal muscle contraction. Additionally TAK-637 inhibits neuronal NK₁ receptors involved in the “local” motor response to stimulation of capsaicin-sensitive primary afferents.

Substance P (SP), neurokinin A, and neurokinin B are a family of small, biologically active peptides, known as tachykinins, which stimulate a wide range of effects through activation of three distinct receptor types designated NK₁, NK₂, and NK₃, respectively. Tachykinins mediate a variety of central and peripheral physiological responses regulating the function of the cardiovascular, respiratory, gastrointestinal, genitourinary, and immune systems (Holzer and Holzer-Petsche, 1997a,b; Quartara and Maggi, 1998). Tachykinins also have pathophysiological effects as proinflammatory mediators and neurotransmitters of peripheral and central pathways responsible for the perception of pain and neurogenic inflammation (Cuello et al., 1993).

In the gastrointestinal tract, SP is expressed in enteric neurons and extrinsic afferent fibers (Costa et al., 1985) and acts as an endogenous ligand binding preferentially to NK₁ receptors in neurons, endothelial cells and endocrine cells within the gastrointestinal epithelium, fibroblasts, smooth muscle, inflammatory, and immune cells. The motor action of tachykinins is associated with activation of NK₁ receptors, causing contractions of intestinal muscles (Holzer and Lemberg, 1980; Maggi et al., 1994) or epithelial secretion (Perdue et al., 1987; Brown et al., 1992; Cooke et al., 1997). SP is implicated in the neurally coordinated control of motility and mucosal secretion (Greenwood et al., 1990), acting as a neurotransmitter often coreleased with acetylcholine (Bartho et al., 1980; Maggi et al., 1994) or epithelial secretion. Functional studies have indicated that NK₁, NK₂, and NK₃ tachykinin receptor types in the intestine are involved in peristaltic reflexes. In general, NK₂ receptors are localized on neurons, whereas NK₁

ABBREVIATIONS: SP, substance P; To, optimal resting tension; TTX, tetrodotoxin; EFS, electrical field stimulation; NANC, nonadrenergic, noncholinergic; DMSO, dimethyl sulfoxide; DR, dose ratio; TAK-637, (αR,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]naphthyridine-6,13-dione; GR 64349, Lys-Asp-Ser-Phe-Val-Gly-R-γ-lactam-Leu-Met-NH₂; GR 73632, H₂N-(CH₂)₄-CO-Phe-Phe-Pro-NMe-Leu-Met-NH₂; MEN-10376, H-Asp-Tyr-o-Trp-Val-o-Trp-o-Trp-Lys-NH₂; CL, confidence limit.
and NK2 receptors are found on muscle cells (Maggi et al., 1993b; Maggi, 1995). However, in the guinea pig ileum, NK1 receptors have been located on inhibitory (Portbury et al., 1996) and excitatory (Southwell et al., 1998) motor neurons and are implicated in activation of enteric reflex circuits activated by villous movement (Southwell et al., 1998). In addition, SP is coexpressed with calcitonin gene-related peptide in the peripheral endings of extrinsic primary afferent fibers. Studies have also shown that SP is functionally involved in the perception of pain (Bueno et al., 1997) and in enterotoxin-induced reflex secretion in the small intestine (Sjoqvist et al., 1993).

Compromised motility in various gastrointestinal disorders is linked to abnormal tachykinin-mediated regulation of the activity of both nerves and muscles involved in enteric and peripheral reflexes. The discovery of tachykinin antagonists, which distinguish between the NK1, NK2, or NK3 tachykinin receptors, presents a more selective therapeutic approach to the normalization of intestinal motility and pain perception. A novel nonpeptide NK1 receptor antagonist TAK-637 [(aR,9R)-7,3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methoxy-6,13-dione] has recently been found to reduce sensory visceral reflexes in guinea pigs (Doi et al., 2000; Kamo et al., 2000) and to reduce stress-related propulsive activity of the colon (Okano et al., 2001). The aim of the present study was to investigate the peripheral action of TAK-637 on NK1 receptor-mediated effects in the gastrointestinal tract. It is well known that NK1 receptors show interspecies heterogeneity; however, there is a close similarity between human and guinea pig receptors (Takeda et al., 1991; Gorbulev et al., 1992) whereas rat NK1 receptors are quite different (Yokota et al., 1989). Thus, we studied the pharmacology of TAK-637 in vitro using intestinal preparations isolated from the guinea pig. Our study provided functional evidence that TAK-637 is a selective antagonist of peripheral NK1 receptors.

Materials and Methods

Experimental Animals. Male Dunkin-Hartley guinea pigs (350–400 g body weight) purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN) were used in the experiments. The animals were housed two per cage under environmentally controlled conditions (21°C, 12-h light/dark cycle) with free access to food and water. All animals were allowed to acclimate to the environment in the animal facility for at least 1 week prior to experiments. The animals were killed by sectioning the carotid arteries under isoflurane anesthesia, and intestinal tissue was harvested immediately. A total of 35 guinea pigs were used in the study. The experimental protocol was approved by the Oklahoma City Veterans Affairs Medical Center Animal Care Committee.

Isometric Contractile Activity in Isolated Intestinal Preparations. Segments of the mid portion of the colon, the ileum (20 cm proximal to the ileocecal junction), or the terminal ileum (5–15 cm proximal to the ileocecal junction), as well as the taenia coli, were isolated and placed in ice-cold Krebs-bicarbonate solution that was continuously aerated with 95% O2 and 5% CO2. Muscle strips (10–12-mm long) containing both muscle layers were dissected from the mucosa-submucosa layer and oriented in the direction of the longitudinal or circular muscle to record isometric contractile activity. Strips (approximately 15-mm long), consisting of the longitudinal muscle with the myenteric plexus attached were dissected from ileal intestinal segments. The taenia coli muscle strips used were 4–to 5-mm long. In one of the experimental series, whole segment preparations (approximately 25-mm long) were dissected from the terminal ileum and mounted vertically for recording of contractile activity in the direction of the longitudinal muscle layer. Each preparation was allowed to equilibrate at zero tension for 20 min, followed by consecutive loading with 0.20-g increments until a level of optimal resting tension (To) was achieved. Resting tension was considered optimal when the contractile response to carbachol (1 μM) or 5-hydroxytryptamine (5-HT, 10 μM) ceased to increase with loading. All experiments were performed at To and isometric contractions were monitored via isometric transducers (Radnoti Glass Technology Inc., Monrovia, CA) and recorded on a Grass-7 polygraph (Grass Instrument Co., Quincy, MA).

Electrical Field Stimulation. Neurally mediated responses were elicited by electrical field stimulation (EFS) applied via pairs of platinum wire electrodes placed parallel to the muscle strips. Submaximal contractile responses, allowing the measurement of both reduction and potentiation of the neurally mediated contractions, were considered an appropriate model to study the effects of TAK-637. Electrical stimuli were generated by a Grass-88 stimulator (Grass Instrument Co.). Contractile responses of isolated longitudinal colonic muscles were evoked by rectangular pulses (0.5 ms pulse duration) applied in 5-s train at 5- to 10-min intervals. Pulse frequencies within the train were increased from 1 to 16 Hz to induce frequency-dependent responses. An electromotor force of 50 V was found to induce submaximal (80–95% of carbachol maximum) contraction of the longitudinal colonic muscle to stimulation frequency of 16 Hz. Consequently, a constant voltage of 50 V was used with all frequencies of stimulation. Identical parameters of stimulation were used to elicit nonadrenergic, noncholinergic (NANC) responses in colonic longitudinal muscles treated with atropine (1 μM) and guanethidine (10 μM). Cholinergic neurally mediated responses were elicited in ileal longitudinal muscle-myenteric plexus strips by single-pulse stimulation (0.5 ms pulse duration) applied at a frequency of 0.1 Hz. Submaximal twitch contractions of the longitudinal muscle were obtained by increasing the voltage by 5-V increments (starting at 10 V) until a maximal response was obtained. The voltage was then reduced by 5 V. Under these conditions, the evoked contractions were equal to 70 to 80% of the maximum.

Experimental Design. Intestinal muscle preparations that functionally express tachykinin NK1, NK2, or NK3 receptors were used to characterize antagonist potency and receptor specificity of TAK-637 effects in the gastrointestinal tract. To achieve equilibrium with NK1 receptors, each concentration of TAK-637 was added into the bath solution 30 min prior to construction of an agonist concentration-response curve or EFS. The effects of TAK-637 on concentration-response curves induced by selective tachykinin receptor agonists were investigated in the following preparations: 1) longitudinal colonic muscle showing NK1 receptor-mediated contractions to [Sar9,Met(O2)11]-SP or GR 64349; 2) circular colonic muscle showing NK1 receptor-mediated contractions to GR 64349 (Maggi et al., 1994); and 3) the taenia coli muscle showing NK1 receptor-mediated contractions to sentkide (Crocio et al., 1995). The blockade of nerve conduction by tetradotoxin (TTX, 1 μM) was used to distinguish between a neuronal and non-neuronal (smooth muscle) site of action of TAK-637. The effects of TAK-637 on neurally mediated responses were evaluated in the longitudinal muscle of the colon. The action of TAK-637 at cholinergic neuromuscular junctions was studied using electrically induced twitch contractions in longitudinal muscle-myenteric plexus strips isolated from the guinea pig ileum. Finally, the effects of TAK-637 were studied in isolated segments of terminal ileum, where capsaicin (10 μM) was applied to stimulate primary afferents and induce local efferent motor responses (Bartho et al., 1982, 1999).

Data Analysis and Statistics. Contractions were measured in millinewtons and normalized for the cross-sectional area of the muscle strip (millimeters squared). The cross-sectional area was calculated as a function of the length of the muscle at To (millimeters), the
wet weight (milligrams), and smooth muscle density of 1.05 mg/mm³ (Gordon and Siegman, 1971). In the experiments designed to study the action of TAK-637 on neurally mediated or capsaicin-induced contractions of isolated intestinal segments, responses were expressed as percentage of the contraction induced by carbachol (1 μM) applied to each preparation at the beginning of the experiment. Differences between the maximal effect of the agonist in the presence and absence of TAK-637 were tested using a paired or unpaired t test, after confirming that the data showed normal distribution with equal variances. Concentration-response curves were constructed in the absence and presence of increasing concentrations of TAK-637 and analyzed fitting the data to a sigmoid dose-response curve by a nonlinear regression using the PRIZM program from GraphPad Software, Inc. (San Diego, CA). The obtained dose ratios (DRs) were further subjected to Schild analysis. An equilibrium dissociation constant (K_D) for TAK-637 was obtained as the antilog of pA_2 derived from the linear regression of mean values of the log (DR−1) plotted against the negative log of TAK-637 concentration. To investigate whether the effect of TAK-637 is neurally mediated, K_D values were estimated in control or TTX-pretreated colonic longitudinal muscles using concentration-response curves to GR 73632 obtained in the absence and presence of a single concentration of 100 nM TAK-637. K_D values based on a single shift of the agonist dose-response curve were calculated by the equation:

\[ K_D + a/(DR - 1) \]

where \( a \) is the antagonist concentration and DR is the ratio of equieffective agonist concentrations (EC_{50}) determined in the presence and absence of the antagonist.

The results were presented as mean ± S.E. Statistical significance was tested using unpaired or paired t test or the nonparametric Mann-Whitney test when appropriate. Differences were considered significant at \( p \) values less than 0.05. Statistical analysis was performed using InStat (GraphPad Software, Inc.).

**Solutions and Drugs.** The modified Krebs-bicarbonate buffer used in the experiments was of the following composition: 120 mM NaCl, 6 mM KCl, 1.2 mM MgCl₂, 1.2 mM NaH₂PO₄, 2.5 mM CaCl₂, 14.4 mM NaHCO₃, and 11.5 mM glucose. The following substances were added to the buffer: 10 μM atropine, 1 μM 5-HT, 1 μM 5-HTP (Sigma), 1 μM 5-HTP (Sigma), 1 μM 5-HTP (Sigma), and stock solutions of 1 mM concentration were stored at −20°C. Working dilutions were prepared fresh for each experiment. TAK-637 and MEN-10376 were dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the bathing solution did not exceed 0.01% and had no significant effect on muscle contractions.

**Results**

**Antagonist Potency of TAK-637 against NK₃ Receptor-Mediated Contractions.** Longitudinal muscles isolated from the guinea pig colon were studied in the presence of atropine (1 μM) to prevent cholinergically mediated contractions. Under the influence of atropine, the preparations would not respond to carbachol; therefore, the optimal tension was adjusted by examining the contractile response induced by 5-HT (10 μM). Under these experimental conditions, the selective NK₃ receptor agonists [Sar⁹, Met(O₂)¹¹]-SP or GR 73632 was added cumulatively at concentrations increasing from 0.1 nM to 1 μM to induce concentration-dependent contractions. The contractile responses showed no tachyphylaxis and were reproducible after

### Table 1

<table>
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<tr>
<th>Treatment</th>
<th>Maximal Contraction</th>
<th>pD₂</th>
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<th>DR</th>
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<tr>
<td></td>
<td>mN/mm²</td>
<td>95% CL</td>
<td>nM</td>
<td></td>
</tr>
<tr>
<td>[Sar⁹, Met(O₂)¹¹]-SP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>7.732</td>
<td>17.7</td>
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<tr>
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<td>7.669</td>
<td>21.4</td>
<td>1.2</td>
</tr>
<tr>
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<td>7.398</td>
<td>40.0</td>
<td>2.3</td>
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<tr>
<td>100 nM</td>
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<td>6.117</td>
<td>665.0</td>
<td>37.5</td>
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<tr>
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<td>7.914</td>
<td>12.1</td>
<td></td>
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<tr>
<td>Control</td>
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<td>7.699</td>
<td>20.0</td>
<td>1.6</td>
</tr>
<tr>
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<td>7.253</td>
<td>55.8</td>
<td>4.6</td>
</tr>
<tr>
<td>100 nM</td>
<td>8.5 (8.5–8.6)</td>
<td>6.487</td>
<td>325.9</td>
<td>26.9</td>
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</table>

Fig. 1. Contractions induced by [Sar⁹, Met(O₂)¹¹]-SP in isolated guinea pig longitudinal colonic muscles pretreated with atropine (1 μM). a, concentration-response curves obtained in the presence of the vehicle (0.1% v/v DMSO) and in the presence of increasing concentrations of TAK-637 (1–100 nM). Contractile responses are expressed in millinewtons normalized per millimeters squared cross-sectional area of the muscle strip. Data points present mean ± S.E.M. of six to eight muscle strips isolated from different guinea pigs. b, Schild plot analysis of the contractile response to [Sar⁹, Met(O₂)¹¹]-SP in the presence of increasing concentrations of TAK-637. DRs are determined from the EC₅₀ values for SP obtained in the presence and absence of TAK-637. Linear regression of the Schild plot yields a straight line and a pA₂ value of 8.325.
washing. However, it was interesting to discover that GR 73632-induced maximal contraction was lower in amplitude compared with \([\text{Sar}^9,\text{Met(O2)}^{11}]\)-SP. Complete concentration-response curves for both \([\text{Sar}^9,\text{Met(O2)}^{11}]\)-SP and GR 73632 were obtained in preparations pretreated for 30 min with the vehicle (DMSO) or increasing concentrations of TAK-637 (1–100 nM). Application of DMSO (0.1% v/v) had no significant effect on spontaneous contractile activity or the concentration-effect curves for \([\text{Sar}^9,\text{Met(O2)}^{11}]\)-SP or GR 73632. TAK-637 showed no changes of spontaneous activity and did not significantly reduce the maximal responses to \([\text{Sar}^9,\text{Met(O2)}^{11}]\)-SP or GR 73632 (Table 1). Nonetheless, TAK-637 induced a concentration-dependent shift of the dose-response curves to higher concentrations of \([\text{Sar}^9,\text{Met(O2)}^{11}]\)-SP (Fig. 1a) and GR 73632 (Fig. 2a). The EC\(_{50}\) values obtained in the absence and presence of TAK-637 are given in Table 1. Schild plot of the rightward shifts of the concentration-effect curves for \([\text{Sar}^9,\text{Met(O2)}^{11}]\)-SP or GR 73632 yielded a straight line (linear coefficient 0.97) with a slope of 1.09 ± 0.14 (95% CL, 0.81–1.23) and a \(K_b\) value of 4.7 nM (Fig. 1b). The Schild analysis of the effect of TAK-637 on GR 73632 responses yielded a straight line (linear coefficient 0.99) with a slope of 0.80 ± 0.08 (95% CL, 0.62–1.12) and a \(K_b\) value of 1.8 nM (Fig. 2b).

A separate set of experiments was performed to establish whether TAK-637 had a neuronal or smooth muscle site of action. The antagonist potency of TAK-637 against contractions induced by GR 73632 was studied in the presence or absence of TTX (1 \(\mu\)M) in colonic longitudinal muscles pretreated with atropine. A single concentration of 100 nM TAK-637 induced similar rightward shifts of the dose-response curves for GR 73632 in the untreated muscles and in the muscles treated with TTX (Fig. 3). The \(K_b\) values of TAK-637 estimated by a single shift of the concentration-response curves obtained in the absence and presence of TTX were 3.7 and 3.5 nM, respectively.

**Receptor Specificity of Effects of TAK-637.** To establish whether TAK-637 is a selective NK\(_1\) receptor antagonist, concentration-response curves for the contractile responses induced by selective activation of NK\(_2\) or NK\(_3\) receptors were studied in the absence and presence of TAK-637. In colonic circular muscle that functionally expresses NK\(_2\) receptors, TAK-637 (100 nM) had no significant effect on the concentration-response curve for the contractile effect of a selective NK\(_2\) receptor agonist, GR 64349 (0.1 nM–1 \(\mu\)M). In contrast, the selective antagonist of NK\(_2\) receptors, MEN-10376 (100 nM), caused a parallel shift of the concentration-response curve to higher concentrations with a DR of 2.6 (Fig. 4a). The taenia coli preparation expresses NK\(_3\) receptors that are activated by the selective NK\(_3\) receptor agonist senktide, causing a contractile response. Pretreatment of the taenia coli with TAK-637 (100 nM) had no significant effect on the concentration-effect curves obtained for senktide (0.1 nM–1 \(\mu\)M) (Fig. 4b).

**Effects of TAK-637 on Neurally Mediated Responses.** In the longitudinal muscle of the colon, EPS (0.5 ms pulse duration, 1–16 Hz pulse frequency, 5-s train duration) induced frequency-dependent responses, which were abolished by TTX (1 \(\mu\)M). Responses to stimulus frequencies of 1 to 4 Hz showed an initial inhibitory component developing during stimulation, which was followed by an “off” contractile response. High frequencies (8–16 Hz) of stimulation induced only contractile responses. Since the inhibitory component depends on the level of resting tension, which was relatively low (0.2–0.5 g/mm\(^2\)) in colonic longitudinal muscle, the effect of TAK-637 was investigated only against the contractile response. TAK-637 (100 nM) did not alter the contractile responses to all frequencies of stimulation (Fig. 5a). In a separate set of experiments, colonic muscles were pretreated with atropine (1 \(\mu\)M) and guanethidine (10 \(\mu\)M), and EPS was applied to induce NANC responses. NANC contractile responses were of lower amplitude, compared with untreated controls, and increased with the increase in stimulus frequency. TAK-637 (100 nM) did not significantly change the NANC contractions, except for responses to the highest stimulus frequencies (8–16 Hz), which showed a modest but significant (\(p < 0.05\)) reduction by TAK-637 (Fig. 5b).

In longitudinal muscle strips isolated from the guinea pig ileum, EPS with single electrical pulses (0.5 ms) applied at a frequency of 0.1 Hz induced twitch-like contractions. These contractions are considered a classical model of cholinergic motor responses since they are abolished by atropine. Treatment with increasing concentrations of TAK-637 (100 nM or...
Effects of TAK-637 on Capsaicin-Induced Contractions. Capsaicin is a sensory neuron stimulant that releases SP and depletes primary sensory afferent neurons of the small nonmyelinated type associated with pain sensation. In isolated segments of terminal ileum, the initial application of capsaicin (10 μM) induced a contractile response that involves the effect of endogenous tachykinins. To avoid the effect of SP depletion, a single application of capsaicin was tested in each preparation. In untreated control preparations, the contractile response to capsaicin was 68 ± 7% of carbachol contraction, whereas pretreatment with TAK-637 (100 nM) completely prevented the development of capsaicin-induced contractions (Fig. 6). In contrast, the response to capsaicin was significantly (p < 0.001) reduced, but not abolished by atropine (1 μM). The development of capsaicin-induced contraction was completely blocked by pretreatment with TTX (1 μM).

Our results demonstrate that TAK-637 has peripheral activity acting as a selective and competitive antagonist of NK1 receptors in the gastrointestinal tract. In the isolated longitudinal muscle of the guinea pig colon, TAK-637 showed high antagonist affinity against contractions induced by [Sar², Met(O²)¹¹]-SP or GR 73632 with Kᵦ values in the nanomolar range. However, as indicated by the Kᵦ values (4.7 nM versus 1.8 nM), the antagonist affinity of TAK-637 was 2.6 times higher when calculated against the contractile responses to GR 73632 compared with [Sar², Met(O²)¹¹]-SP contractile responses. Such a difference in the affinity of a single antagonist is not uncommon when comparing the effects of an NK₁ receptor antagonist against C-terminal truncated (GR 73632) and full-length ([Sar², Met(O²)¹¹]-SP) analogs of SP (Patacchini et al., 1995; Jenkinson et al., 1999). In addition, although GR 73632 and [Sar², Met(O²)¹¹]-SP had similar potency (EC₅₀ values were 12.1 and 17.0 nM, respectively) in the longitudinal colonic muscle, the amplitude of the maximal response to GR 73632 was about 2 times higher than the response to [Sar², Met(O²)¹¹]-SP. Taken together, the results suggest that TAK-637, similar to other nonpeptide antagonists (Longmore et al., 1994; Zeng and Burcher, 1994; Patacchini et al., 1995), may show variable affinity for a single NK₁ receptor population, depending on the agonist with which it competes. In the broader context of drug-receptor interaction, these discrepancies suggest that GR 73632 and
[Sar9,Met(O2)11]-SP bind differentially to the NK1 receptor in the nanomolar range.

In addition to inducing a direct contractile effect, tachykinins have been shown to regulate intestinal activity via neuronal NK2 (Zagorodnyuk et al., 1995), NK3 (Maggi et al., 1993a), and NK1 (Johnson et al., 1998; Lecci et al., 1999) receptors. In the current study, experiments were designed to differentiate between the neuronally mediated and direct effects of TAK-637. The DR and $K_0$ calculated for a single shift of the dose-response curves to GR 73632 produced by TAK-637 (100 nM) in colonic longitudinal muscles pretreated with TTX were similar to the respective values obtained in untreated preparations. These findings imply that in the colonic longitudinal muscle, TAK-637 antagonizes the effect of a non-neuronal NK1 receptor. Moreover, we have demonstrated that TAK-637 is a selective antagonist interacting with NK1 but not NK2 or NK3 receptors. TAK-637 was ineffective against the contractile responses of circular colonic muscle to a NK2 receptor agonist GR 64349 or against the NK2 receptor-mediated contractions of the taenia coli induced by senktide. In summary, TAK-637 was defined as a competitive antagonist interacting selectively with smooth muscle NK1 receptors causing contraction in the guinea pig colon.

Electrical field stimulation was used to further characterize the peripheral action of TAK-637 in the gastrointestinal tract by investigating its effect on neuromuscular motor responses. However, despite the fact that TAK-637 antagonizes the effect of exogenous SP in the longitudinal muscle of the colon, a significant inhibitory effect of TAK-637 was found only against NANC contractile responses induced by high frequencies of EFS. Also, the cholinergic twitch contractions induced by EFS in the longitudinal muscle-myenteric plexus strips were unaffected by TAK-637. The discrepancy between the effect of TAK-637 on responses induced by exogenous SP and the lack of a complete inhibitory effect on motor neurally mediated responses could be explained by the method of intramural nerve stimulation. Responses elicited by EFS are the net effect of stimulation of all intramural nerve terminals sensitive to the applied stimulus, and the muscle response reflects exclusively the immediate release of neurotransmitters at the neuroeffector, i.e., neuromuscular junction. Thus, the negative results obtained with TAK-637 suggest that TAK-637 has no effect on the motor neurotransmission at cholinergic and NANC neuromuscular junctions, at which SP is not an immediate neurotransmitter. A small portion of the contraction in response to NANC stimulation at higher frequency was blocked by TAK-637, indicating that TAK-637 may selectively inhibit the component of neuromuscular motor responses that is mediated by NK1 receptors.

A capsaicin-stimulated component of intestinal sensory-motor reflexes is maintained in the isolated intestine and was used as a model to study the effects of TAK-637. In the guinea pig ileum, capsaicin stimulates primary afferent nerve terminals, which in turn activate cholinergic (and to a smaller extent noncholinergic) motor neurons, causing a net contractile response (Bartho et al., 1982). Since the capsaicin-induced contraction was followed by a rapid desensitization of capsaicin-sensitive sensory neurons, a single response...
was induced in either control or TAK-637-pretreated segments of terminal ileum. The striking lack of response in TAK-637-pretreated preparations compared with the presence of contractile response in vehicle-treated control preparations clearly indicated that TAK-637 acts as an inhibitor of a sensory-motor response in the ileum, which requires an intact NK1 receptor mediated pathway. However, the source of receptive input to the capsaicin-sensitive fibers, i.e., the functional role of the reflex(es) that could be modulated by TAK-637, requires further investigation. In addition, we found that cholinergic motor neurons blocked by atropine are responsible for a major portion of the capsaicin-induced contractile response, since the capsaicin-induced contraction was reduced by approximately 80% in the presence of atropine. A small portion (approximately 20%) of the contractile responses to capsaicin, which was also inhibited by TAK-637, is due to the effect of a noncholinergic motor transmitter(s) released simultaneously with acetylcholine. The nature of the excitatory noncholinergic response was not identified in the present experiments. In contrast to a recent finding by Bartho et al. (1999), we did not find a “nontachykininergic” component of the capsaicin-induced excitatory response of the small intestine. The ability of TAK-637 to inhibit capsaicin-induced response supports the suggestion that NK1 receptor antagonists have a therapeutic potential to inhibit effects mediated by enteric neurons and to block local sensory responses at the periphery. These effects of TAK-637 may be of particular benefit in the treatment of functional gastrointestinal disorders.

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References


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