Desensitization of the peristaltic reflex induced by mucosal stimulation with the selective 5-HT\textsubscript{4} agonist tegaserod

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Grider, John R. Desensitization of the peristaltic reflex induced by mucosal stimulation with the selective 5-HT\textsubscript{4} agonist tegaserod. Am J Physiol Gastrointest Liver Physiol 290: G319–G327, 2006. First published October 13, 2005; doi:10.1152/ajpgi.00326.2005.—The intestinal peristaltic reflex induced by mucosal stimulation is mediated by mucosal release of serotonin (5-HT), which acts on 5-HT\textsubscript{4} receptors located on CGRP-containing afferent nerve terminals. Exposure of the colonic mucosa to the 5-HT\textsubscript{1} receptor agonist tegaserod in the range of 1 nM to 10 \textmu M elicits a peristaltic reflex and stimulates colonic propulsion. The present study was designed to identify the 5-HT\textsubscript{4} receptor subtype mediating the reflex and determine whether functionally effective concentrations of tegaserod desensitize the reflex induced by mucosal stimulation. Exposure of rat colonic mucosa to tegaserod in the range of 5 nM to 5 \textmu M for 5 or 10 min caused rapid time- and concentration-dependent desensitization of the peristaltic reflex induced by mucosal stroking, consistent with the operation of a rapidly desensitizing 5-HT\textsubscript{4b} receptor subtype. Desensitization was accompanied by a decrease in CGRP release. The rate of recovery of peristaltic response depended on the desensitizing concentration of tegaserod: ascending contraction and descending relaxation recovered within 15 min after 5–50 nM tegaserod, 30 min after 0.5 \textmu M, and 60 min after 5 \textmu M. Neither CGRP release nor the peristaltic reflex induced by muscle stretch was affected by 5-HT\textsubscript{4} receptor desensitization, providing further evidence that 5-HT does not mediate the reflex induced by muscle stretch. These results suggest in cases of increased 5-HT availability or prolonged exposure, such as colitis, that it is likely the peristaltic reflex will be blunted.

enteric nervous system; myenteric plexus; colon; serotonin receptors; calcitonin gene-related peptide; gastrointestinal motility

THE CLASSIFICATION OF SEROTONIN (5-HT) RECEPTORS IS CONTINUOUSLY EVOLVING. SEVEN CLASSES OF RECEPTORS, 5-HT\textsubscript{1} THROUGH 5-HT\textsubscript{7}, EACH SUBDIVIDED INTO NUMEROUS SUBTYPES, HAVE BEEN IDENTIFIED. EXCEPT FOR 5-HT\textsubscript{3} RECEPTORS, WHICH ARE LIGAND-GATED ION CHANNELS, ALL OTHER CLASSES OF RECEPTORS APPEAR TO BE COUPLED TO ONE OR MORE HETEROTRIMERIC G PROTEINS (3, 21, 27). A VARIABLE NUMBER OF 5-HT\textsubscript{4} RECEPTOR SUBTYPES HAVE BEEN IDENTIFIED AS HETEROGENEOUS AMONG DIFFERENT SPECIES AND TISSUES. HUMAN 5-HT\textsubscript{4} RECEPTORS ARE SUBDIVIDED INTO AT LEAST SIX SUBTYPES BASED ON THEIR EFFECTS ON PHARMACOLOGICAL RESPONSES (1, 3, 4).

5-HT\textsubscript{4} RECEPTOR SUBTYPES ARE FOUND IN THE BRAIN AND IN TWO MAJOR PERIPHERAL LOCATIONS: THE MUSCULARIS TUNICA OF THE ARTRIUM, WHERE ONLY 5-HT\textsubscript{4a} RECEPTORS ARE PRESENT, AND THE INTESTINE, WHERE 5-HT\textsubscript{4b} RECEPTORS ARE THE MAJOR PERIPHERAL LOCATIONS WHERE 5-HT\textsubscript{4} RECEPTORS ARE EXPRESSED. HUMAN 5-HT\textsubscript{4} RECEPTORS ARE SUBDIVIDED INTO AT LEAST SIX SUBTYPES BASED ON THEIR EFFECTS ON PHARMACOLOGICAL RESPONSES (1, 3, 4).

Desensitization of 5-HT\textsubscript{4} receptor function was induced by exposure of the mucosa to tegaserod, an indole carboxaldehyde derivative, over a range of concentrations between 1 nM and 10 \textmu M. The rate of recovery of peristaltic response depended on the desensitizing concentration of tegaserod: ascending contraction and descending relaxation recovered within 15 min after 5–50 nM tegaserod, 30 min after 0.5 \textmu M, and 60 min after 5 \textmu M. Neither CGRP release nor the peristaltic reflex induced by muscle stretch was affected by 5-HT\textsubscript{4} receptor desensitization, providing further evidence that 5-HT does not mediate the reflex induced by muscle stretch. These results suggest in cases of increased 5-HT availability or prolonged exposure, such as colitis, that it is likely the peristaltic reflex will be blunted.

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lowed by measurement of ascending contraction and descending relaxation and CGRP release.

**MATERIALS AND METHODS**

**Measurement of peristaltic reflex in compartmented flat sheet segments of rat colon.** The peristaltic reflex was measured in a 5-cm segment of the middle to distal rat colon, which was opened to form a flat sheet and pinned mucosal side up in a three-compartment organ bath as described in detail previously (17, 19). The compartments were separated by vertical partitions sealed with vacuum grease, and each contained 2 ml of a Krebs bicarbonate medium. The medium contained (in mM) 118 NaCl, 4.8 KCl, 1.2 KH2PO4, 2.5 CaCl2, 1.2 MgSO4, 25 NaHCO3, and 11 glucose with 10 μM amastatin, 1 μM phosphoramidon, and 0.1% bovine serum albumin. Stimuli designed to evoke the peristaltic reflex were applied to the central compartment. These consisted of 1) stroking the mucosa with a fine brush (2–8 strokes at a rate of 1 stroke/s), 2) radial muscle stretch applied via a hook-and-pulley assembly (6 and 10 g for 10 s each), and 3) by the addition of the selective 5-HT4 agonist tegaserod (1 μM) for 2 min. Ascending contraction of circular muscle was measured in the orad compartment and descending relaxation in the caudad peripheral compartment using force-displacement transducers attached to the muscle layers.

**Desensitization of peristaltic response with tegaserod.** After measurement of control responses, tegaserod was added at various concentrations (5, 50, 500, and 5,000 nM) to the central compartment for a period of 5 or 10 min. A separate colonic preparation was used with each concentration of tegaserod. After a given concentration of tegaserod was washed out, fresh Krebs bicarbonate medium was added and the peristaltic reflex in response to mucosal stroking, radial muscle stretch, or readdition of tegaserod was remeasured immediately (time 0). In experiments involving mucosal stroking, the reflex was measured again at 5, 15, 30, or 60 min. In each experiment, only one mucosal stimulus was applied (i.e., 2, 4, 6, or 8 strokes) using different colonic preparations.

**Measurement of CGRP release.** Control CGRP release was measured in experiments during which stimuli were applied to the central compartment for a period of 15 min. In separate experiments, muscle stretch (6 or 10 g) and mucosal stroking (4 or 8 strokes) were applied at intervals of 3 min for a period of 15 min. In control experiments with tegaserod, the agonist (1 μM) was applied for 5 min. The medium was collected for measurement of control CGRP release. For each preparation separately, 5 μM tegaserod was then applied for 10 min to induce desensitization, and the medium discarded and replaced with fresh Krebs bicarbonate. The response to each stimulus was then remeasured immediately as described above. The medium was collected again for measurement of CGRP release.

CGRP was measured by radioimmunoassay as described previously using antibody RAS 6006 (17). The limit of detection of the assay was 2.6 fmol/ml, and the IC50 was 31.5 ± 1.3 fmol/ml of original sample. The antibody reacts with rCGRP and hCGRP but does not cross react with tegaserod, calcitonin, amylin, substance P, neurokinin A, neurokinin B, somatostatin, VIP, or [Met]enkephalin.

**Data analysis.** Ascending contraction and descending relaxation were measured as grams force and expressed as the percent of control response obtained with a maximal stimulus (8 strokes or 10-g stretch). Values were calculated as means ± SE of measurements obtained in n experiments. For measurement of desensitization and recovery of the peristaltic reflex, the response to a single stimulus was measured. The tissue was exposed to a single concentration of tegaserod for 10 min followed by washout, and the stimulus was repeated at 5, 15, 30, and 60 min. A separate animal was used for each stimulus and each concentration of tegaserod. Thus n represents the number of experiments and animals for each curve. Statistical significance was evaluated using ANOVA and Student’s t-tests (GraphPad Software, San Diego, CA). The half-time (t1/2) for recovery from desensitization was calculated as the time for the response to recover one-half from the maximal desensitization response at a given level of stimulation and desensitizing concentration of tegaserod.

CGRP was measured as femtomoles per milliliter of sample, normalized to the wet weight of colonic tissue in the central compartment, and expressed as femtomoles per 100 mg per minute. Separate animals were used for each experiment, and separate experiments were done for each stimulus. Thus n represents the number of experiments and animals. Statistical significance was evaluated using ANOVA and Student’s t-tests (GraphPad Software, San Diego, CA).

**Materials.** CGRP, CGRP antiserum RAS 6009, and 125I-labeled CGRP were purchased from Bachem-Peninsula (Torrance, CA). Tegaserod (HTF-919) was a gift from Drs. D. Romer and H.-J. Pfannkuche, Novartis (Basel, Switzerland). All other chemicals and agents were purchased from Sigma (St. Louis, MO).

**RESULTS**

**Desensitization of the peristaltic reflex with tegaserod.** As shown previously (14), the addition of the selective 5-HT4 agonist tegaserod (1 μM) to the central compartment elicited both components of the peristaltic reflex: ascending contraction (0.9 ± 0.1 g) and descending relaxation (0.6 ± 0.1 g) of circular muscle in the orad and caudad peripheral compartments, respectively. When the mucosa in the central compartment was first exposed to 5 μM tegaserod for 5 min, the subsequent response to 1 μM tegaserod measured immediately after washing was decreased by 65% (ascending contraction: 0.33 ± 0.07 g; descending relaxation: 0.21 ± 0.04 g). Exposure to tegaserod for 10 min abolished subsequent responses to 1 μM tegaserod (ascending contraction: 0.06 ± 0.04 g; descending relaxation: 0.04 ± 0.02 g). These results confirm the ability of tegaserod to desensitize the 5-HT4 receptor that initiates the peristaltic reflex.

**Cross desensitization of the peristaltic reflex induced by mucosal stimuli by prior exposure to tegaserod.** Exposure of the mucosa in the central compartment to 5 μM tegaserod for 5 or 10 min caused a decline in the reflex response to mucosal stroking recorded in the peripheral compartments that was reversible (Fig. 1). The cross desensitization was not unexpected because previous studies (20) had shown that the release of 5-HT elicited by mucosal stroking initiated a peristaltic reflex by activating 5-HT4 receptors on sensory nerve terminals. The extent of decline in ascending contraction and descending relaxation caused by prior exposure to tegaserod depended on the duration of exposure and the strength of the

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**Fig. 1.** Effect of tegaserod desensitization on the peristaltic reflex. Tracings of ascending contraction and descending relaxation elicited by 6 mucosal brush strokes were recorded in the peripheral orad and caudad compartments of a 3-compartment preparation of rat colon. For this tracing, control responses were obtained in the absence of desensitization, immediately after exposure of the mucosa in the central compartment to tegaserod (5 μM) for 5 and 10 min and following repeated washing of the preparation with fresh Krebs buffer.
mucosal stimulus (Fig. 2A). Exposure of the mucosa to tegaserod for 5 min abolished the response to the lowest stimulus (2 strokes) and significantly inhibited the response to the highest stimulus (41 ± 6% inhibition of ascending contraction and 51 ± 7% inhibition of descending relaxation). Exposure of the mucosa to tegaserod for 10 min was more effective, abolishing the peristaltic reflex elicited by two and four mucosal strokes and nearly abolishing the highest response (91 ± 9% inhibition of ascending contraction and 81 ± 5% inhibition of descending relaxation). However, exposure of the mucosa for 5 or 10 min to tegaserod had no effect on ascending contraction or descending relaxation elicited by 6 or 8 g of muscle stretch (Fig. 2B). This was consistent with our previous study indicating that muscle stretch did not elicit 5-HT release or activate 5-HT3 receptors (20).

Dependence of desensitization on the concentration of tegaserod. The degree of inhibition of ascending contraction or descending relaxation measured immediately after 10-min exposure to tegaserod depended on the desensitizing concentration of tegaserod (Fig. 3). Ascending contraction and descending relaxation elicited by two strokes were abolished by prior exposure of the mucosa to all concentrations of tegaserod, including the lowest concentration of 5 nM. Ascending contraction in response to the highest stimulus (8 strokes) was inhibited by 35 ± 12% (P < 0.01) after prior exposure to 5 nM and abolished, as noted above, after prior exposure to 5 μM tegaserod. Similarly, descending relaxation in response to eight strokes was inhibited by 26 ± 7% (P < 0.01) after prior exposure to 5 nM tegaserod and strongly inhibited (81 ± 5%), as noted above, after prior exposure to 5 μM tegaserod.

Recovery of the peristaltic reflex after exposure to tegaserod. The recovery of the peristaltic reflex after 10-min exposure to tegaserod is depicted in Figs. 4 and 5. As noted in MATERIALS AND METHODS, a control response to a single stimulus (e.g., 2 strokes) was first measured. Then, the mucosa was exposed to one concentration of tegaserod (e.g., 5 nM) for 10 min. The agent was rapidly washed out, and the response to the same stimulus was tested immediately after (time 0) and again at 5, 15, 30, and 60 min. In a separate preparation, the same sequence was repeated except that the mucosa was exposed to a different concentration of tegaserod (e.g., 50 nM). This approach allowed construction of the curves shown in Figs. 4 and 5 to demonstrate the rate of recovery of response.

After exposure of the mucosa to 5 or 50 nM tegaserod, the response to all stimuli (2–8 strokes), whether measured as ascending contraction or descending relaxation, recovered rapidly reverting to between 85 and 100% of control response within 15 min. After exposure to 500 nM tegaserod, responses recovered less rapidly, reverting to between 80 and 100% of control response within 30 min. After exposure to 5 μM
tigaserod, recovery of response to ~90% of control required ~60 min. A similar pattern of recovery was noted when the $t_{1/2}$ for recovery was compared for different levels of stimulation and different desensitizing concentrations of tigaserod (Table 1). For desensitizing concentrations of 5 and 50 nM tigaserod, the $t_{1/2}$ for recovery was rapid, ranging from 1 to 6 min for ascending contraction and descending relaxation induced by all levels of stimulation (2–8 strokes). At the higher desensitizing concentration of tigaserod, it is noteworthy that there was a dichotomy between the responses to lower and higher levels of

Fig. 3. Dependence of desensitization on the concentration of tigaserod. Mucosal strokes (2–8 strokes) were applied sequentially to the central compartment of a 3-compartment preparation of rat colon before and immediately after exposure of the mucosa for 10 min to various concentrations of tigaserod (5, 50, and 500 nM and 5 μM). Separate colonic preparations were used for each concentration. Results are expressed as % maximal control response (ascending contraction: 0.9 ± 0.1 g; descending relaxation: 0.6 ± 0.1 g). Values are means ± SE of 5–9 experiments.

Fig. 4. Recovery of ascending phase of the peristaltic reflex after exposure to tigaserod. A: control response to a single stimulus (e.g., 2 strokes) was first measured. The mucosa in the central compartment was then exposed to one concentration of tigaserod (e.g., 5 nM) for 10 min. The agent was rapidly washed out, and the response to the same stimulus was tested immediately after (time 0) and again at 5, 15, 30, and 60 min. In separate preparations, the same sequence was repeated except that the mucosa was exposed to a different concentration of tigaserod (e.g., 50 and 500 nM or 5 μM). Similar series of experiments were done using 4 (B), 6 (C), or 8 strokes (D) and the same range of concentrations of tigaserod. The results were expressed as % control response to each stimulus (i.e., 2, 4, 6, or 8 strokes) before exposure to tigaserod. Data are means of 4 experiments with each stimulus and each concentration; thus A–D represent the results from 16 separate preparations.
stimulation. The responses to the lower levels of stimulation (2 and 4 strokes) were completely desensitized, remained completely desensitized longer, and took longer to recover ($t_{1/2}$ on the order of 20–40 min). In contrast, at the higher desensitizing concentrations of tegaserod, the response to higher levels of stimulation were only partially desensitized, did not remain desensitized for extended periods of time, and recoved more rapidly ($t_{1/2}$ on the order of 11–19 min).

**Release of CGRP after exposure to tegaserod.** Previous studies had shown that the addition of 5-HT or tegaserod to the mucosa or endogenous release of 5-HT by mucosal stimulation activates 5-HT$_4$ receptors on CGRP-containing sensory neurons (18, 20). Experiments were therefore done to determine whether prior exposure of the mucosa to tegaserod caused a change in subsequent CGRP release by mucosal stimuli or by reexposure to tegaserod. Control measurements of CGRP release were obtained in different preparations in response to the addition of 10^{-6} M tegaserod for 5 min and in response to mucosal stimulation (4 or 8 strokes) or muscle stretch (6 and 10 g). The mucosa was then exposed to 5 x 10^{-6} M tegaserod for 10 min; the medium was washed out, and the effect of various stimuli (1 $\mu$M tegaserod, mucosal stroking, or muscle stretch) was determined. Basal CGRP release was not affected by exposure to tegaserod (4.8 ± 0.4 fmol·100 mg$^{-1}$·min$^{-1}$ before tegaserod vs. 3.9 ± 0.3 fmol·100 mg$^{-1}$·min$^{-1}$ after tegaserod). However, after exposure to 5 $\mu$M tegaserod, CGRP release induced by mucosal stroking or by readdition of tegaserod was significantly inhibited (Fig. 6). In contrast, CGRP release induced by muscle stretch was not affected by prior exposure to tegaserod, providing further confirmation that CGRP release induced by muscle stretch is not mediated by 5-HT.

![Fig. 5. Recovery of the descending phase of the peristaltic reflex after exposure to tegaserod. The same preparations used to depict the results in Fig. 4 were also used to measure simultaneously descending relaxation. The sequence and number of experiments is identical to that described in Fig. 4. A: 2 strokes; B: 4 strokes; C: 6 strokes; D: 8 strokes.](http://ajpgi.physiology.org/)

**Table 1. Rate of recovery following desensitization**

<table>
<thead>
<tr>
<th>Desensitizing Concentration</th>
<th>Response</th>
<th>Stimulus Strokes</th>
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<tbody>
<tr>
<td>5 nM</td>
<td>AC</td>
<td>2 min 3 min 3 min 1 min</td>
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<tr>
<td></td>
<td>DR</td>
<td>4 min 6 min 3 min 4 min</td>
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<tr>
<td>50 nM</td>
<td>AC</td>
<td>9 min 5 min 6 min 7 min</td>
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<tr>
<td></td>
<td>DR</td>
<td>8 min 6 min 7 min 6 min</td>
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<tr>
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<td>AC</td>
<td>23 min 12 min 10 min 10 min</td>
</tr>
<tr>
<td></td>
<td>DR</td>
<td>20 min 12 min 11 min 13 min</td>
</tr>
<tr>
<td>5 $\mu$M</td>
<td>AC</td>
<td>42 min 29 min 12 min 17 min</td>
</tr>
<tr>
<td></td>
<td>DR</td>
<td>41 min 25 min 19 min 14 min</td>
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Values are half-times. AC, ascending contraction; DR, descending relaxation.
release of 5-HT, which, in turn, activates 5-HT4 receptors. Administration of an agonist tegaserod for 5 or 10 min causes a decrease in the rate of CGRP release was measured as described in MATERIALS AND METHODS. In separate experiments, tegaserod (1 μM), mucosal stroking (4 or 8 strokes), and muscle stretch (6 or 10 g) were applied in the central compartment, and the medium was obtained for measurement of CGRP release. The mucosa was then exposed to 5 μM tegaserod for 10 min. After the medium was replaced, the same stimuli were reapplied and the medium was collected for measurement of CGRP. Results are means ± SE of 4 separate experiments with each stimulus. **P < 0.01 from control.

DISCUSSION

Exposure of colonic mucosa to the selective 5-HT4 receptor agonist tegaserod for 5 or 10 min causes a decrease in the magnitude of the peristaltic reflex elicited by mucosal stimulation, providing further evidence that mucosal stimuli induce release of 5-HT, which, in turn, activates 5-HT4 receptors mediating the peristaltic reflex. The desensitizing effect of tegaserod was detectable at concentrations as low as 5 nM, increasing with higher concentrations up to 5 μM. A previous study (18) had shown that the addition of tegaserod to the mucosa elicited ascending contraction and descending relaxation with an EC50 of 2–5 nM; maximal response was obtained with 0.1–1 μM. Similarly, intraluminal perfusion of isolated colonic segments with tegaserod increased the velocity of propulsion of fecal pellets with an EC50 of 7 nM (22). Maximal increase in propulsive activity was observed at concentrations of 0.1–1 μM. Considering that in human studies (2, 7), about two-thirds of the oral dose of tegaserod is excreted unchanged in the feces, it is likely that the colonic mucosa is exposed to concentrations similar to that used in the present study. This also suggests that addition of tegaserod to the mucosa as in the present study is likely to reflect a major route by which the colon lumen is exposed to tegaserod after oral administration. Thus the present study shows that these functionally effective concentrations of tegaserod delivered to the mucosa can lead to desensitization of the reflex for variable intervals.

Recovery from desensitization occurred rapidly, within 5–15 min after exposure to low concentrations of tegaserod (5–50 nM). A longer period, ~30 min, was required for recovery after exposure to 0.5 μM, a concentration that by itself is capable of eliciting a maximal peristaltic response (18). Examination of the rate of recovery supports the notion that the site of desensitization is the intrinsic sensory neuron rather than ascending or descending interneurons or motoneurons. The t1/2 values for recovery are very similar for the ascending contraction and descending relaxation response to a given level of stimulation at each descending concentration of tegaserod. This would be expected if desensitization occurred at the intrinsic sensory neuron that is common to activation of both the ascending and descending pathways. This also suggests that it is unlikely that desensitization occurred at the level of the myenteric 5-HT-containing neurons because this project only in the caudal direction. In contrast to the peristaltic reflex initiated by mucosal stimulation, even exposure to the highest concentration of tegaserod (5 μM), which virtually abolishes the response to mucosal stroking, had no effect on the peristaltic reflex elicited by muscle stretch, providing further evidence that the effect of muscle stretch activates a separate population of sensory neurons that are extrinsic neurons and that the response of these sensory neurons is not mediated by 5-HT-dependent activation of 5-HT4 receptors (19, 20).

After exposure to 5 μM tegaserod, CGRP release induced by mucosal stroking or reapplication of tegaserod was inhibited by ~80%. CGRP release induced by muscle stretch was not affected. Previous studies had shown that CGRP release induced by muscle stretch was derived from extrinsic sensory neurons with cell bodies in the dorsal root ganglia and was not mediated by 5-HT (17, 19). CGRP release induced by mucosal stroking or short-chain fatty acids was mediated by 5-HT acting on 5-HT4 receptors located on intramural CGRP-containing neurons.

The pattern of desensitization by tegaserod is consistent with inactivation of a rapidly desensitizing 5-HT4 receptor subtype, probably 5-HT4b (i.e., r5-HT4L) (1, 10) This notion is supported by a recent study demonstrating that 5-HT4b is the main 5-HT4 receptor subtype expressed in intestinal tissues, although this study did not distinguish among nerve, muscle, glial, or other cell types in the intestine (30). A similar pattern of rapid desensitization of response was previously observed on exposure of dispersed human intestinal smooth muscle cells to tegaserod (25). In these cells, the desensitization was reflected by a decrease in cAMP formation and muscle relaxation. Blockade of cAMP-dependent protein kinase activity with H-89 prevented tegaserod-induced desensitization, suggesting that desensitization of 5-HT4 receptors in smooth muscle cells was mediated by PKA-induced phosphorylation of the receptor. The mechanism of 5-HT4 receptor desensitization involved in the peristaltic reflex could not be determined in the present study.

Although the results suggest that desensitization of the reflex was mediated by desensitization of 5-HT4 receptors, it is useful to discuss alternative interpretations. Tegaserod has recently been shown to have binding affinities to the 5-HT2B receptor that are similar to its binding affinity for the h5-HT4(c) receptor stably expressed in HEK-293 cells (4). Pharmacological studies in a number of in vitro and in vivo preparations indicate that tegaserod acts as an antagonist at the 5-HT2B receptor rather than an agonist as it does at the 5-HT4 receptor. Thus it is possible that the therapeutic effect of tegaserod may involve a composite of the effects mediated by activation of 5-HT4 receptors and inhibition of 5-HT2B receptors. It is, however, unlikely that the effects of tegaserod in the present study are the result of an interaction with the 5-HT2B receptor because antagonists do not activate intracellular signaling pathways and interactions of antagonists with membrane receptors are gen-

Fig. 6. CGRP release induced by mucosal stroking and muscle stretch before and after exposure of the mucosa to tegaserod. Basal and stimulated CGRP release was measured as described in MATERIALS AND METHODS. In separate preparations, tegaserod (1 μM), mucosal stroking (4 or 8 strokes), and muscle stretch (6 or 10 g) were applied in the central compartment, and the medium was obtained for measurement of CGRP release. The mucosa was then exposed to 5 μM tegaserod for 10 min. After the medium was replaced, the same stimuli were reapplied and the medium was collected for measurement of CGRP. Results are means ± SE of 4 separate experiments with each stimulus. **P < 0.01 from control.
eraly not thought to lead to desensitization. A second possible explanation is that prior exposure to tegaserod may have depleted CGRP from sensory neurons to such an extent that CGRP release was decreased on reactivation of 5-HT4 receptors. As shown in Fig. 6, prior exposure to tegaserod resulted in a decrease of CGRP release induced by mucosal stroking or reapplication of tegaserod. It could also be argued that the extent of depletion was proportional to the time of exposure as well as to the initial concentration of tegaserod. It seems unlikely, however, that brief exposure to low concentrations such as 5 or 50 nM, which is followed by rapid recovery, would cause sufficient depletion of CGRP as to affect subsequent responses, and it seems more likely that the rapid recovery of response reflects reactivation of a desensitized receptor. In a previous study (18), we had shown that allowing an interval of 45 min to pass between applications of a maximal concentration of tegaserod (1 μM) did not affect successive responses.

Independent of the mechanism of desensitization, the results of this study have important implication to the effect of altered levels of endogenous 5-HT in pathology and to the clinical use of tegaserod. Whereas exposure of the mucosa to concentrations of tegaserod in the range of 5–50 nM induces transient desensitization of the peristaltic reflex that did not exceed 15 min, exposure to higher concentrations leads to prolonged desensitization. This is the first study to our knowledge to characterize the desensitization of the peristaltic reflex and to show that it is likely due to desensitization of the 5-HT4 receptor on the CGRP-containing primary afferent neurons of the colon.

The desensitization of the peristaltic reflex, as shown in the present study, is likely to explain the desensitization of the propulsion of intraluminal contents described in several other studies. In earlier studies using the guinea pig model of colonic pellet propulsion, Wade et al. (40) showed that intraluminal perfusion with the endogenous ligand 5-HT induced desensitization of propulsive activity. In addition, they showed that blockade of the SERT with fluoxetine caused depletion of CGRP as to affect the initial concentration of tegaserod. It seems unlikely, however, that brief exposure to low concentrations such as 5 or 50 nM, which is followed by rapid recovery, would cause sufficient depletion of CGRP as to affect subsequent responses, and it seems more likely that the rapid recovery of response reflects reactivation of a desensitized receptor. In a previous study (18), we had shown that allowing an interval of 45 min to pass between applications of a maximal concentration of tegaserod (1 μM) did not affect successive responses.

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The desensitization of the peristaltic reflex, as shown in the present study, is likely to explain the desensitization of the propulsion of intraluminal contents described in several other studies. In earlier studies using the guinea pig model of colonic pellet propulsion, Wade et al. (40) showed that intraluminal perfusion with the endogenous ligand 5-HT induced desensitization of propulsive activity. In addition, they showed that blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine had similar effects by increasing endogenous 5-HT; low levels of fluoxetine blockade of the SERT with fluoxetine induced desensitization of the 5-HT4 receptor and release of CGRP by the intrinsic sensory neurons that mediate the reflex. This is the first study to our knowledge to characterize the desensitization of the peristaltic reflex and to show that it is likely due to desensitization of the 5-HT4 receptor on the CGRP-containing primary afferent neurons of the colon.

The effects of prolonged activation, desensitization, and recovery of the 5-HT4 receptor in the peristaltic reflex described in the present study may also explain the results of studies demonstrating alternating diarrhea and constipation in animal models where endogenous 5-HT is elevated as a result of the absence or depression of the enteric SERT. In knockout mice that lack SERT, Chen et al. (9) noted that increased motility and watery diarrhea alternated with periods of decreased motility and constipation. The authors interpreted this pattern as resulting from increased activation of 5-HT receptors followed by desensitization of 5-HT receptors. These changes in propulsion are consistent with the changes in the peristaltic reflex described in the present study.

The results of the present study demonstrating desensitization of the peristaltic reflex and the release of CGRP by the intrinsic primary afferent neurons following exposure to 5-HT4 agonist have pathophysiological implications based on recent studies of colitis. Studies by Linden et al. (29) demonstrated decreased SERT, increased cell enterochromaffin (EC) number, and increased 5-HT in the mucosa of guinea pigs in which colitis had been induced with trinitrobenzene sulfonic acid. The result of the enhanced and prolonged availability of endogenous 5-HT was a reduced velocity of propulsion of fecal pellets and decreased sensitivity to 5-HT receptor antagonism, which the authors attributed to the likely desensitization of 5-HT receptors. A similar increase in EC cell density and 5-HT content was found to occur in rats in which dextran sodium sulfate was used to induce colitis, although motility was not measured in this study by Oshima et al. (34). A recent study by O’Hara et al. has suggested (33) that desensitization of the 5-HT receptors may also play a role in altered intestinal motility. TNBS-induced ileitis in the guinea pig was accompanied by increased EC cell numbers, decreased SERT, and enhanced release of 5-HT in response to chemical stimulation of the mucosa, thus raising the possibility of 5-HT desensitization mediating changes in intestinal motility. Thus the present study demonstrates the characteristics of the desensitization and recovery of the underlying peristaltic reflex is likely to be important in understanding the effects of altered serotonin and SERT levels on propulsion in experimental models of colitis.

Similar patterns of constipation and diarrhea and changes in 5-HT and SERT have been reported to accompany inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) in humans, although there is some variability in findings. Moses et al. (32) demonstrated decreased SERT staining in human rectal mucosal biopsies from patients with ulcerative colitis and constipation-predominant IBS compared with rectal biopsies from normal controls and patients with diarrhea-predominant IBS, whereas Dunlap et al. (13, 14) have found impaired 5-HT release in constipation-predominant IBS where as postinfectious IBS patients demonstrated increased postprandial 5-HT release and EC cell number. A recent study by Coates et al. (11) showed decreased SERT immunoreactivity in ulcerative colitis and IBS patients, although in this study 5-HT levels were reduced. These authors postulate that the decreased SERT could lead to initial enhancement of the reflex activity and that with continued exposure to 5-HT, there would be a decrease in the peristaltic reflex due to receptor desensitization. Our present study confirms this notion and demonstrates that exposure to increased concentrations or durations of a 5-HT4 agonist desensitizes the peristaltic reflex and release of CGRP from the primary sensory neurons that mediate the reflex. Other studies have demonstrated that not only are the levels of SERT altered in IBS, but also that SERT polymorphisms tended to be associated with changes in clonic transit in patients with IBS (8). In the study by Yeo et al. (41), diarrhea-predominant IBS was associated with the expression of the homogenous short phenotype of SERT, which has a reduced functional 5-HT transport capacity. Thus differences in the
expression phenotypes of SERT could lead to the elevation of 5-HT to levels that cause desensitization of 5-HT4 receptors. Because the peristaltic reflex is similar in human and animal models and 5-HT4 receptors play a critical role in initiating the peristaltic reflex in humans (16, 18), it is likely to unaltered luminal tegaserod acts to limit the response to oral that desensitization of the mucosal peristaltic reflex in response are on the order of 10−15% above placebo (7). It is possible present study. The estimates of therapeutic gain with tegaserod sensitization of the peristaltic reflex similar to that seen in the lower intestine and colon that could blunt the prevails in the lower intestine and colon that could blunt the propulsion of the small intestine and colon that could blunt the response to tegaserod by inducing rapid and long-lasting desensitization of the peristaltic reflex similar to that seen in the present study. The estimates of therapeutic gain with tegaserod are on the order of 10−15% above placebo (7). It is possible that desensitization of the mucosal peristaltic reflex in response to unaltered luminal tegaserod acts to limit the response to oral tegaserod in patients in a manner similar to that shown in rats in the present study.

GRANTS

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REFERENCES


