Serotonin (ie, 5-HT), a ubiquitous neurotransmitter, exerts its action by interacting with 7 receptor subtypes. In the intestinal tract, 5-HT mediates a variety of physiologic effects on enterocytes, smooth muscle cells, and enteric neurons through its effects on several 5-HT receptor subtypes that regulate gastrointestinal tract motility, vascular tone, and secretion. Serotonergic signaling abnormalities have also been putatively implicated in the pathogenesis of functional intestinal diseases. In humans, 5-HT receptor subtypes are established targets for drugs used in the treatment of CNS disorders (eg, mental disorders) as well as gastrointestinal disorders (eg, gastroesophageal reflux, functional dyspepsia, irritable bowel disease, and functional constipation) and for the prevention of nausea. Although activation of several different receptors is responsible for 5-HT-mediated responses within the gastrointestinal tract, the 5-HT₄ receptor subtype is considered particularly important, both physiologically and pathophysiologically. Activation of 5-HT₄ receptor agonists have gastrointestinal prokinetic activities. In equine veterinary medicine, the application of sero-
tonergic drugs is primarily limited to modulation of the peristaltic activity of the gastrointestinal tract. The evaluation of possible targets for the development of new prokinetic drugs for use in horses is emerging because gastrointestinal disorders, such as postoperative ileus, are common causes of death in horses and often involve considerable economic losses.10

Despite the fact that a variety of prokinetic drugs is on the market, none of those drugs are considered sufficient in terms of efficacy and safety. Cisapride, a 5-HT4 receptor agonist that has 5-HT3 and dopamine receptor (D2) antagonistic properties, has proven effective to improve impaired gastrointestinal tract motility in horses.11 In 2000, cisapride was withdrawn from the market because of adverse prolongation of the QT interval in humans.12 In addition, the partial 5-HT4 agonist tegaserod—a drug that is highly effective in humans with constipation-predominant irritable bowel disease—was withdrawn in 2007 because of concerns regarding an increased incidence of myocardial infarction, angina, and stroke in humans, although the mechanism of adverse effects is still unclear and the adverse effects were not 5-HT4 receptor related. Despite the risk potential of the aforementioned compounds, 5-HT4 receptors remain promising targets in the treatment of gastrointestinal tract motility disorders. A therapeutic value of tegaserod in horses has been suggested by recently published reports.13–15 In vitro, tegaserod increases intestinal smooth muscle contraction in specimens of the pelvic flexure (a hairpin turn in the large colon that is predisposed to obstruction) of horses.15,16 Moreover, it was demonstrated in vivo that the 5-HT4 partial agonist tegaserod accelerates the gastrointestinal transit time and increases the frequency of defection and scores of intestinal sounds in healthy horses.17 Therapeutic plasma concentrations of tegaserod were achieved following oral administration of a single dose of 0.27 mg/kg.14 However, controversial findings regarding the general impact of 5-HT4 receptors on equine gastrointestinal tract motility have been reported.16,18 Delesalle et al.16 found a lack of evidence for the presence of 5-HT4 receptors in the small intestine of horses, their conclusion was based on the observation that selected 5-HT4 receptor antagonists could not alter serotonin-induced tonic contractions of equine jejunal tissue in vitro.

Besides tegaserod, other 5-HT4 receptor ligands have been investigated with respect to their potential for treatment of gastrointestinal tract motility disorders in humans and horses. One of the most relevant of them is prucalopride, which is anticipated to be released soon onto the European market for the treatment of chronic constipation in humans. In addition to proven efficacy across different patient groups, prucalopride provides a favorable cardiovascular and overall safety profile.17 Partial 5-HT4 receptor agonists such as PF-0135408218 or CJ-03346619 that are currently in early stages of pharmaceutical development are expected to exert a favorable effect on gastrointestinal motor disorders with reduced adverse effects mediated by other related receptors.

Efficient gastrointestinal tract motility requires the coordinated activity of several cell types including enteric nerves, smooth muscle cells, and ICCs.20 Intestinal cells of Cajal are oval or stellate cells of mesenchymal origin. They are widely distributed in the smooth muscle layers of the gastrointestinal tract. The ICCs are predominantly located between the circular and longitudinal muscle layers as well as in the deep muscular plexus. Their cytoplasm is scarce but extends to make contacts with autonomic nerve fibers of the myenteric plexus through varicose nerve terminals and to establish gap junctions with adjacent smooth muscle cells. The ICCs have a key role in the regulation of intestinal peristalsis by generating spontaneous, rhythmical electrical oscillations (called slow waves) and by mediating enteric motor neurotransmission and afferent signaling. The ICCs mediate gastrointestinal tract motility through cholinergic excitatory and nitrergic inhibitory motor neurotransmissions.20–22 Interstitial cells of Cajal have been detected in circular muscle layer and the myenteric plexus of horses.23 Their pacemaker function, in terms of generating slow waves, is essential for gastrointestinal tract motility.20

Given that differing distributions of 5-HT4 receptors along the gastrointestinal tract in different species might explain the different responses of either smooth muscle contraction or relaxation, the purpose of the study reported here was to evaluate the expression of the 5-HT4 receptor subtype and investigate the modulating function of those receptors on intestinal contractility in selected intestinal tissues obtained from horses. Furthermore, the effect of the 3-HT4 partial agonist tegaserod alone or in combination with serotonin on contractility of intestinal specimens was investigated. Our investigations were focused on tissues at 3 locations in the intestinal tract: the duodenum, ileum, and pelvic flexure. The duodenum and ileum were selected for investigation because the small intestine is frequently associated with gastrointestinal tract disorders in horses. In addition, pelvic flexure was chosen because of its established motility pacemaker activity, which regulates colonic aboral and retropropulsive transit of digesta.24 Also, we evaluated the expression and possible colocalization of 5-HT4 receptors and c-kit by use of a double-labeling immunofluorescence technique. The c-kit marker is a tyrosine kinase receptor that is a well-established marker of ICCs.

Materials and Methods

Tissue samples and preparation—Specimens of duodenum, ileum, and pelvic flexure were obtained from 24 horses with no history or signs of gastrointestinal tract disease after they had been slaughtered at local abattoirs. The horses (males and females aged 1 to 30 years) were of various breeds, and specimens were collected within 15 minutes after the horses were slaughtered by use of a captive bolt.

In each horse, 2 to 6 tissue specimens (including all muscle layers) were obtained from a location 20 cm aboral to the pylorus (duodenum), from the proximal insertion site of the plica ileocaecalis (ileum), and from the pelvic flexure at the position where tenia were no longer evident. Subsequently, the intestinal content was disposed of by rinsing of the specimens with cooled (5°C) KH solution (NaCl, 118.4 mM; KCl, 4.7 mM;
Immunostaining procedures for detection of 5-HT4 receptors in horses was selected on the basis of protein sequence data deduced from coding regions extracted from the Horse Genome Project sequences and from the equine HTR4 partial cDNA sequence obtained by our group from an equine colon sample (GenBank accession No. AY263357). A commercial affinity-purified polyclonal antibody against the third cytoplasmic domain (aa 214-260) of the human 5-HT4 receptor was used. The third cytoplasmic domain of the human protein differs only by 4 residues along the same region of the horse 5-HT4 receptor (91% identity; data not shown). To investigate the possible colocalization of 5-HT4 receptors and c-kit on ICCs, the affinity-purified polyclonal goat anti-mouse stem cell factor receptor (SCF R)/c-kit antibody was used.

Strips of intestinal tissue (approx 15 mm in length and 5 mm in width) obtained from each location in each horse were prepared, pinned on foam plates, and flash frozen in liquid nitrogen. Tissues were cryostat sectioned at a thickness of 5 μm. The immunofluorescence staining procedure was performed at room temperature (approx 20°C), and PBS solution was used to wash sections thoroughly between each step. Briefly, the slides were fixed in 2% paraformaldehyde in PBS solution for 30 minutes, treated with 0.2M glycine in PBS solution for 20 minutes. After washing for 5 minutes 3 times, sections were allowed to equilibrate for 30 minutes. The bath solution was routinely set to a tension of 1 g and the initial muscle tension was readjusted after 60 minutes. The tension was readjusted after 60 minutes. The specimen was examined for spontaneous activity. Only specimens that had constant frequency, amplitude, and basal tone of contraction for at least 10 minutes prior to the start of the experiment were used for analysis. Variables such as basal tone or amplitude at this time were designated as predrug values. Cumulative concentration-response curves following treatment with 5-HT in the absence or presence of tegaserod were generated. At 5-minute intervals, the concentration of 5-HT in the organ bath was increased logarithmically (from 10⁻³ to 10⁻¹M). For experiments performed in the presence of tegaserod, preparations were preincubated for 20 minutes with tegaserod at a concentration of 1 × 10⁻⁶M. Subsequently, 5-HT was added as described. Solvent control experiments (by use of methylpyrrolidone for tegaserod and H₂O for 5-HT) were conducted similarly.

Experimental protocols—Intestinal specimens were obtained as described. The intestinal specimens were kept in cooled KH solution aerated with carbon (95% O₂ and 5% CO₂) until used in organ bath experiments.

**Immunofluorescence experiments**—The most adequate antibody for detection of 5-HT₄ receptors in horses was selected on the basis of protein sequence data deduced from coding regions extracted from the Horse Genome Project sequences and from the equine HTR4 partial cDNA sequence obtained by our group from an equine colon sample (GenBank accession No. AY263357). A commercial affinity-purified polyclonal antibody against the third cytoplasmic domain (aa 214-260) of the human 5-HT₄ receptor was used. The third cytoplasmic domain of the human protein differs only by 4 residues along the same region of the horse 5-HT₄ receptor (91% identity; data not shown). To investigate the possible colocalization of 5-HT₄ receptors and c-kit on ICCs, the affinity-purified polyclonal goat anti-mouse stem cell factor receptor (SCF R)/c-kit antibody was used.

Strips of intestinal tissue (approx 15 mm in length and 5 mm in width) obtained from each location in each horse were prepared, pinned on foam plates, and flash frozen in liquid nitrogen. Tissues were cryostat sectioned at a thickness of 5 μm. The immunofluorescence staining procedure was performed at room temperature (approx 20°C), and PBS solution was used to wash sections thoroughly between each step. Briefly, the slides were fixed in 2% paraformaldehyde in PBS solution for 30 minutes, treated with 0.2M glycine in PBS solution for 20 minutes, and made permeable in 0.3% Triton X-100 for 20 minutes. Sections were then undergone immunostaining procedures for detection of 5-HT₄ receptors and c-kit. The sections were washed for 5 minutes 3 times in PBS solution with 0.1% Tween 20 before being blocked with 5% donkey serum with streptavidin for 1 hour at room temperature, according to the manufacturer’s protocol. Primary antibodies (rabbit anti-human 5-HT₄ polyclonal antibody diluted 1:200 [5 ng/μL] and goat anti-mouse SCF R/c-kit polyclonal antibody diluted 1:25 [4 ng/μL]) supplemented with biotin were applied overnight at room temperature. After washing for 5 minutes 3 times, sections were incubated with secondary antibodies (biotin-SP–coupled donkey anti-rabbit IgG (diluted 1:3,000) and Cy2-labeled donkey anti-goat IgG (diluted 1:100) for 1 hour at room temperature. Further incubation with (Cy3-labeled streptavidin diluted 1:3,000) was performed for 1 hour at room temperature. Sections were treated with 4',6-diamidino-2-phenylindole to stain nuclei. All immunolabeling reagents were diluted in an antibody diluent.

After immunostaining, the slides were washed in PBS solution and covered with mounting medium.
were generated by a stimulator. After EFS was initiated, the specimens were checked for activity (amplitude of contractions). To evoke submaximal contractions, the current intensity was gradually increased every 5 minutes until contractility was initiated. To generate a concentration-response curve for tegaserod and the corresponding solvent methylpyrrolidone, only specimens for which constant frequency, amplitude, and basal tone of contraction were maintained for at least 10 minutes prior to the start of the experiment were selected. At 5-minute intervals, tegaserod was added in half-logarithmic steps reaching concentrations ranging from $10^{-10}$ to $10^{-6}$M. Solvent controls were conducted similarly.

Data and statistical analyses—The contractility variables of basal tone (in g), $A_{max}$ (in g), and frequency (in counts/min [for spontaneous contractions]) were recorded in 5-minute intervals. The data acquisition of contractility variables started 5 minutes prior to the first addition of 5-HT or tegaserod (designated as the predrug period) and ceased 5 minutes after the last addition. All data are reported relative to the values obtained during the predrug period.

The data collected over the period of cumulative addition of 5-HT or tegaserod followed by 5-HT were examined by use of a Friedman test for each experimental protocol and orientation of the muscle layers separately. Observed effects for all variables and all experiments over time were compared with the effect of solvent by use of an ANOVA for repeated measures followed by the Bonferroni multiple comparison test. Values are expressed as mean ± SEM; a value of $P < 0.05$ was considered significant.

Concentration-response curves for 5-HT and tegaserod plus 5-HT were calculated by use of the Hill function, and estimation by use of the least squares method was applied. The underlying equation for the Hill function is:

$$\text{Response} = \frac{V_{max} \times C^\alpha}{(C^\alpha + K^\alpha)}$$

where $C$ is the compound concentration, $K$ represents the EC$_{50}$ value, and the exponent $\alpha$ describes the shape of the function.

Significance of comparisons made on the basis of this model was determined by use of the likelihood ratio statistic, which yields a $\chi^2$ test. Standard deviations reported for variables in the Hill model are based on the Cramer-Rao statistic. The results were expressed as $V_{max}$ and EC$_{50}$ of $A_{max}$.

Results

Immunofluorescence experiments—In all enteric locations examined, marked c-kit immunoreactivity was detected between circular and longitudinal muscle layers in the myenteric plexus (Figure 1). Strong 5-HT$_4$ receptor immunoreactivity was observed in the walls of blood vessels of the myenteric region, but not in ICCs because no colocalization of 5-HT$_4$ receptors with c-kit was identified. Furthermore, weak to moderate 5-HT$_4$ immunoreactivity was detected in both intestinal muscle layers (Figure 2). Nevertheless, staining was more pronounced in the circular muscle layer than in the

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Figure 1—Representative photomicrographs illustrating the localization of 5-HT$_4$ receptors and c-kit by use of immunofluorescence staining performed on cryostat sections of duodenum, ileum, and pelvic flexure obtained from 2 horses. For each tissue type, findings for 1 experimentally treated section (columns marked Exp) and 1 control section (columns marked Con) are provided. Experimentally treated sections were double labeled with anti–c-kit and anti–5-HT$_4$ receptor antibodies, and Cy2- and Cy3-tagged streptavidin were used to detect c-kit (green fluorescence; top-row images) and 5-HT$_4$ receptors (red fluorescence; middle-row images), respectively. In matching control sections, primary antibodies were omitted. For the experimentally treated and control sections for each tissue type, top- and middle-row images were merged to create the bottom-row images. Notice that merged images revealed no colocalization of 5-HT$_4$ and c-kit immunoreactivity. CM = Circular muscle layer. LM = Longitudinal muscle layer. MP = Myenteric plexus. Bar = 100 µm (applies to all images).
longitudinal muscle layer. Moderate 5-HT₄ immunoreactivity was observed in the mucosal region, where crypts are expected to be located, and strong staining was observed in round-shaped areas within the mucosal villi (Figure 3). Submucosal blood vessels were also highly stained for the 5-HT₄ receptor, and the immunofluorescence signals were assigned to the tunica media and tunica intima. The negative controls were devoid of specific immunolabeling.

**Organ bath experiments**—Intestinal tissue preparations that had regular contractile activity 10 minutes

Figure 2—Representative photomicrographs illustrating the localization of 5-HT₄ receptors by use of immunofluorescence staining performed on 2 cryostat sections of ileum obtained from 2 horses. Findings for 1 experimentally treated section (column marked Exp) and 1 control section (column marked Con) are provided. The experimentally treated section was labeled with anti-5-HT₄ receptor antibody and Cy3-labeled streptavidin; primary antibody was omitted in the control section. Differential interference contrast microscopy was used to observe structural details in the tissue sections (top-row images). In the middle-row images, receptor localization is indicated by red fluorescence. For each section, top- and middle-row images were merged to create the bottom-row images. In the merged image for the experimentally treated section, expression of 5-HT₄ receptors is localized in longitudinal and circular muscle layers as well as in myenteric blood vessels. Bar = 200 µm (applies to all images). See Figure 1 for key.
before the start of the experiment were included in the study. In total, 38 duodenal preparations, 42 ileal preparations, and 52 pelvic flexure preparations were collected from 13 horses. Overall, 27 of 38 (71%) duodenal preparations, 29 of 42 (69%) ileal preparations, and 31 of 52 (60%) pelvic flexure preparations met the study inclusion criterion. With regard to the spontaneous contractions at the start of the experiment of duodenal, ileal, and pelvic flexure specimens, mean frequency was 11.48, 7.18, and 3.75 counts/min, respectively, and mean Amax was 1.46, 1.14, and 2.35 g, respectively; basal tone was 1.08, 1.13, and 1.23 g, respectively.

In the experiments involving spontaneous contractions, treatment with 5-HT caused concentration-dependent increases in basal tone in duodenal and ileal tissue strips, compared with the effect of solvent, whereas no similar effect on basal tone was evident in pelvic flexure preparations (Figure 4). Significant (P < 0.001) differences between 5-HT–treated duodenal specimens and solvent controls were evident at 5-HT concentrations of 10⁻⁷ to 10⁻⁵ M. In preparations of duodenum, the shape of the concentration-response curve was biphasic with a first plateau at a concentration of 10⁻⁷ M and a maximum effect at 10⁻⁵ M. A biphasic concentration-response curve was also obtained for ileal preparations with a first plateau at concentrations of 10⁻⁸ to 10⁻⁶ M followed by increases in basal tone at higher concentrations (10⁻⁴ to 10⁻³ M).

Preincubation of tissue preparations with tegaserod resulted in decreases in basal tone in all intestinal specimens, compared with the effect of 5-HT alone, although the change was significant only for specimens from the duodenum at 5-HT concentrations of 10⁻⁷ and 10⁻⁵ M (Figure 4). In preparations from the ileum and pelvic flexure, tegaserod did not significantly change basal tone with respect to the effect of 5-HT alone.

Treatment with 5-HT at all concentrations except 10⁻⁷ M resulted in significant (P < 0.05) increases (as a percentage of the predrug period value) in Amax in pelvic flexure specimens, compared with the effect of solvent, whereas no significant increases in Amax were detected...
in preparations from the duodenum and ileum (Figure 5). Exposure to tegaserod did not change \( A_{\text{max}} \) values, compared with the effect of 5-HT alone, in specimens from any intestinal location.

Analysis of the \( EC_{50} \) values for basal tone induced by treatment with 5-HT with or without preincubation with tegaserod revealed no differences among intestinal locations. Regardless of experimental conditions, values of \( V_{\text{max}} \) for basal tone were significantly (\( P < 0.001 \)) higher in specimens from the duodenum and ileum, compared with specimens from the pelvic flexure.

Compared with specimens exposed to 5-HT and tegaserod, the \( V_{\text{max}} \) of basal tone induced by 5-HT alone was significantly higher in preparations of duodenum (all values of \( P < 0.01 \)) and ileum (all values of \( P < 0.05 \)), but not in preparations of pelvic flexure (Table

Figure 4—Effect (determined in organ bath experiments) of 5-HT on basal tone in longitudinal specimens of duodenum (\( n = 9 \); A), ileum (10; B), and pelvic flexure (10; C) obtained from 24 horses following preincubation of specimens for 20 minutes without (black circles) or with (inverted black triangles) tegaserod (1 \( \times 10^{-6} \)M). Following the preincubation period (60 minutes), the concentration of 5-HT in the organ bath was increased logarithmically (from \( 10^{-10} \) to \( 10^{-5} \)M); control specimens were preincubated without tegaserod and exposed only to solvent (H\(_2\)O [white circles]). Data are reported as mean basal tone (as a percentage of the predrug period value) and SEM. *Response to 5-HT alone (without tegaserod preincubation) is significantly (\( P < 0.05 \)) different from the corresponding response to solvent. †Response to 5-HT following tegaserod preincubation is significantly (\( P < 0.05 \)) different from the corresponding response to 5-HT alone. ‡Significant (\( P < 0.05 \)) effect over time in this treatment group.

Figure 5—Effect (determined in organ bath experiments) of 5-HT on \( A_{\text{max}} \) of spontaneous contractions in longitudinal specimens of duodenum (\( n = 9 \); A), ileum (10; B), and pelvic flexure (10; C) obtained from 24 horses following preincubation of specimens for 20 minutes without (black circles) or with (inverted black triangles) tegaserod (1 \( \times 10^{-6} \)M). Following the preincubation period (60 minutes), the concentration of 5-HT in the organ bath was increased logarithmically (from \( 10^{-10} \) to \( 10^{-5} \)M); control specimens were preincubated without tegaserod and exposed only to solvent (H\(_2\)O [white circles]). Data are given as mean \( A_{\text{max}} \) (as a percentage of the predrug period value) and SEM. See Figure 4 for remainder of key.
Table 1—Mean $V_{\text{max}}$ and $EC_{50}$ values of the basal tone (determined in organ bath experiments) in specimens of duodenum, ileum, and pelvic flexure obtained from 24 horses following preincubation of specimens for 20 minutes without or with tegaserod (1 $\times$ 10$^{-6}$M [Teg]).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Treatment</th>
<th>No. of experiments</th>
<th>$V_{\text{max}}$ (g [95% confidence interval])</th>
<th>$EC_{50}$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>5-HT</td>
<td>8</td>
<td>0.79$^a$ (0.36–1.72)</td>
<td>4.1 $\times$ 10$^{-5}$</td>
</tr>
<tr>
<td></td>
<td>5-HT and Teg</td>
<td>8</td>
<td>0.47$^a$ (0.09–2.60)</td>
<td>5.6 $\times$ 10$^{-7}$</td>
</tr>
<tr>
<td>Ileum</td>
<td>5-HT</td>
<td>10</td>
<td>1.05$^a$ (0.01–165.5)</td>
<td>9.2 $\times$ 10$^{-4}$</td>
</tr>
<tr>
<td></td>
<td>5-HT and Teg</td>
<td>10</td>
<td>0.15(7.43 $\times$ 10$^{-6}$–2.86 $\times$ 10$^{-7}$)</td>
<td>1.00 $\times$ 10$^{-5}$</td>
</tr>
<tr>
<td>Pelvic flexure</td>
<td>5-HT</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>5-HT and Teg</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = Not applicable (ie, 5-HT did not induce an effect on spontaneous contractions).

$^a$Within a column, values with the same superscript are significantly ($P < 0.05$) different from each other.

1. Analysis of $EC_{50}$ and $V_{\text{max}}$ values for $A_{\text{max}}$ did not reveal any significant differences among the intestinal preparations, regardless of experimental conditions (data not shown). Neither 5-HT nor tegaserod treatment affected the frequency of contractions in tissue preparations from any of the 3 intestinal locations.

EFS data—Preincubation with tegaserod did not significantly change basal tone in preparations from any of the intestinal locations, compared with the effect of solvent (data not shown). In all preparations investigated, tegaserod induced a significant and concentration-dependent increase in $A_{\text{max}}$ over time (duodenum and ileum, $P < 0.001$; pelvic flexure, $P < 0.05$; Figure 6).

Compared with the effect of solvent, values of $A_{\text{max}}$ were significantly ($P < 0.05$) increased in duodenal preparations at tegaserod concentrations of 10$^{-7}$ through 10$^{-4}$M and in pelvic flexure preparations at all but 1 tegaserod concentration in the range of 10$^{-9}$ through 10$^{-4}$M.

In EFS tissue preparations, the values of $EC_{50}$ and $V_{\text{max}}$ for basal tone did not differ among intestinal locations. With regard to $A_{\text{max}}$, the value of $V_{\text{max}}$ for the duodenal specimens was significantly ($P < 0.05$) lower than the value for the ileal or pelvic flexure preparations; the values for the ileal and pelvic flexure preparations did not differ significantly (Table 2).

Discussion

The results of the present study indicate that 5-HT receptors are present in the duodenum, ileum, and pelvic flexure of horses. The distribution of 5-HT$_4$-immunoreactive cells was consistent throughout the 3 regions of the gastrointestinal tract examined. Although 5-HT$_4$ receptor immunoreactivity was detected in the circular and longitudinal muscle layers of the intestine, 5-HT$_4$ receptors were not present in the myenteric plexus. These observations are in agreement with the results of Streutker et al, who identified 5-HT$_4$ receptors by use of immunohistochemical analysis and in situ hybridization in specimens of human nonneuronal cells in the gastrointestinal tract. In that study, 5-HT$_4$ receptors were localized in the circular and longitudinal layer of the tunica muscularis, whereas neurons did not stain specifically for 5-HT$_4$ receptors. However, in specimens of small intestine from guinea pigs and colon from rats and mice, 5-HT$_4$ receptor immunoreactivity was associated with intrinsic primary afferent neurons and motor neurons in the myenteric ganglia. Use of other detection methods, such as autoradiography, has revealed that neuronal 5-HT$_4$ receptor expression is detectable not only in colonic tissue from guinea pigs but also in colonic tissue from humans, even though myenteric localization in human tissue was less distinct than that in guinea pig tissue. In agreement with our findings, Streutker et al also detected 5-HT$_4$ receptors in blood vessels. The strong immunoreactivity in the blood vessels could indicate an important role of 5-HT$_4$ receptors in the regulation of mesenteric blood flow in horses. Among species, 5-HT$_4$ receptor expression and function in blood vessels is variable. Cocks and Arnold reported that relaxation of isolated pulmonary veins obtained from sheep is mediated via activation of 5-HT$_4$ receptors, but there was no evidence for 5-HT$_4$-mediated relaxation in dog, pig, or human pulmonary veins. Furthermore, 5-HT$_4$ receptor mRNA has been detected via reverse transcriptase PCR analysis in rat blood vessels but not in vascular tissues from pigs. In cultured human endothelial cells from pulmonary and coronary arteries, umbilical vein, and aorta, the same group reported weak expression of 5-HT$_4$ receptor mRNA. In contrast, results of functional and radioligand-binding experiments involving isolated intrapulmonary arteries and veins obtained from humans did not provide evidence for the involvement of 5-HT$_4$ receptors in vasoconstriction, and in pulmonary arteries obtained from rabbits, 5-HT$_4$ receptor mRNA could not be detected.

In the present study, 5-HT$_4$ receptor immunoreactivity was evident in the tunica intima and tunica media of enteric blood vessels. This finding is in agreement with findings of other studies in humans in which 5-HT$_4$ receptors were detected on endothelial cells and vascular smooth muscle cells. Currently, the potential impact of the presence of 5-HT$_4$ receptors in blood vessels on modulation of gastrointestinal tract motility has not been investigated, to our knowledge.

A recent study performed by our group revealed that ganglion cells of the myenteric plexus of horses react with antibodies against c-kit, thereby providing evidence that ICCs were present in all intestinal locations tested (the same locations as those investigated in the present study). The ICCs are considered important for gastrointestinal tract motility because of their role as electrical pacemakers, generating spontaneous electrical slow waves that propagate to smooth muscle cells to evoke phasic contractions. In contrast to the findings...
for 5-HT, receptors in horses.34 results of the present study indicated that 5-HT7 receptors are not colocalized with the ICC marker c-kit. In other studies27,35 in mice and guinea-pigs, 5-HT4 receptors were expressed by myenteric ICCs.

The expression of 5-HT4 receptors in equine intestinal mucosa raises the question whether these receptors have a role in mucosal secretion. In the rat colon as well as in guinea pig and human ileum, the transmucosal short-circuit current response to 5-HT is mediated by a receptor of the 5-HT4 type.36–38 In rats and mice, it has been reported39,40 that 5-HT4 receptors are involved in secretion of bicarbonate from the duodenal mucosa, which is crucial to maintain mucosal integrity. Although Safsten et al39 postulated an exclusively myenteric location of 5-HT4 receptors on the basis of data obtained from the duodenum of rats, the findings of a study in mice by Tuo et al40 indicated that 5-HT4 receptor mRNA is present in duodenal mucosa, which supports our observations in equine intestinal tissue. The impact of mucosal 5-HT, receptors in secretory processing in horses has to be investigated further. In humans, expression of 5-HT4 receptors in the mucosa of the duodenum was greater than expressions in mucosa of the stomach, which indicates a prominent role of serotoninergic signaling at the mucosal level in this part of the intestine.41 Possible pathways include the arachidonic acid pathway and activation of sensory dendrites in the lamina propria, which leads to a sensory reflex arc.42

In the present study, increasing concentrations of 5-HT were applied to spontaneously contracting intestinal tissue specimens from horses, and concentration-dependent increases in basal tone in duodenal and ileal preparations were observed. Interestingly, this effect of 5-HT was not evident in pelvic flexure specimens. Because there is no information about the distribution of 5-HT receptor subtypes within the equine gastrointestinal tract, it is not possible to find plausible explanations for this observation. What is known, however, is that 5-HT immunoreactivity varies among intestinal locations in adult horses; the levels of 5-HT immunoreactivity in duodenum and ileum are twice as high as the level in the large intestine.43 In guinea pigs, 5-HT and tegaserod cause the same maximum stimulation in colon peristalsis.44 Data from an in vitro study45 involving rectal tissue from dogs indicated that tegaserod induces 55% of the maximum response induced by 5-HT, which is in agreement with the results of the present study. Most available data indicate that tegaserod is a potent partial 5-HT4 receptor agonist. Nevertheless, it has recently been reported46 that tegaserod blocks 5-HT2B receptors as well. Because of their mixed-drug–specific and tissue-dependent properties, 5-HT4 agonists are able to express tissue selectivity (ie, behave as partial agonists in some tissues and as full agonists in other tissues).47 In fact, agonist properties of tegaserod against recombinant receptors and receptors in isolated tissue preparations differ,48 although the extent to which these findings impact the clinical efficacy of tegaserod as a

Table 2—Mean Vmax and EC50 values of the Amax of EFS-induced contractions (determined in organ bath experiments) in specimens of duodenum, ileum, and pelvic flexure obtained from 24 horses following treatment of specimens with tegaserod at concentrations ranging from 10−10 to 10−6 M.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of experiments</th>
<th>Vmax (g [95% confidence interval])</th>
<th>EC50 (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>12</td>
<td>0.74±0.03 (0.06–9.61)</td>
<td>1.5 × 10−10</td>
</tr>
<tr>
<td>Ileum</td>
<td>14</td>
<td>0.93±0.03 (0.10–8.80)</td>
<td>9.46 × 10−10</td>
</tr>
<tr>
<td>Pelvic flexure</td>
<td>6</td>
<td>1.08±0.03 (0.13–8.74)</td>
<td>2.16 × 10−9</td>
</tr>
</tbody>
</table>

See Table 1 for key.
prokinetic agent remains to be determined. Results of a previous study18 of samples of small intestine obtained from horses are in concordance with findings of the present study; 5-HT increased the basal tone in a concentration-dependent fashion when administered as single doses of various concentrations. However, when serotonin was added to longitudinal jejunal preparations in a cumulative manner (10⁻¹⁰ to 10⁻⁴M) in that study,18 a bell-shaped concentration-response curve was generated, whereas we did not encounter a desensitization problem over time in our study. Similarly, in another previous study,69 desensitization of equine jejunal specimens did not occur as a result of cumulative administration of 5-HT. In the present study, preincubation of tissue preparations from the small intestine with tegaserod resulted in a significant decrease in basal tone, compared with the effect of 5-HT alone. In vitro experiments in guinea-pig ileum, tegaserod revealed an intrinsic activity of 0.2, which corresponds to a fifth of that possessed by the full agonist 5-HT.54 On the basis of that finding, tegaserod can induce a submaximal tissue response (acting as a partial agonist) and may competitively block the effect of serotonin (acting as a full agonist), causing a decrease in contraction activity. In duodenal preparations, the effect of tegaserod on basal tone at different 5-HT concentrations was significant.

The results of the EFS experiments involving cumulative application of tegaserod also indicated that tegaserod has the functional characteristics of a partial agonist. The EFS protocol was set up to induce submaximal contractions, therefore, the amplitude of contractions could be augmented or decreased via application of tegaserod to the organ bath. Tegaserod caused an increase in Amax (compared with the effect of solvent) in equine intestinal specimens from all 3 locations. These observations are at least partly conflicting with results obtained in a previous study13 in which the ability of tegaserod to initiate contraction activity in preparations of ileum and pelvic flexure from horses was investigated. In that study,13 tegaserod increased the incidence of contractions in pelvic flexure specimens, but had no effect on contractility in ileal specimens. The discrepancy in findings between that investigation and the present study might be due to a difference in study design; in 1 study, the initiation of spontaneous contractility in noncontracting specimens was assessed, and in the other, modulation of the amplitude of existing spontaneous contractions was investigated. The prokinetic effect of tegaserod in equine pelvic flexure specimens is supported by data reported by Delco et al.14

On the basis of the results of the present study, it appears that the effect of tegaserod depends on the contractile status of the tissue. The partial 5-HT, agonist caused both attenuation of 5-HT-induced contractions and stimulation of submaximal contractions elicited by EFS. The pharmacological properties of partial agonists might be exploited to develop effective promotility drugs.53 The features of tegaserod render it a promising candidate for treatment of gastrointestinal tract motility disorders in horses,52 particularly because acceleration in gastrointestinal transit in healthy horses following administration of tegaserod has been reported recently.15 Moreover, because of their drug-specific and tissue-related properties, 5-HT, receptor agonists are tissue selective.65 Given the variable effects of selective compounds that target the different 5-HT receptor subtypes, an evaluation of the structure, function, and occurrence of 5-HT receptor subtypes within the gastrointestinal tract of horses could provide better understanding of the intestines’ location-dependent differences in serotonin response.

References