Ca\(^{2+}\) channel blockade by verapamil inhibits GMCs and diarrhea during small intestinal inflammation

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Lee, Ching-Wen, Sushil K. Sarna, Chandar Singaram, and Margaret A. Casper. Ca\(^{2+}\) channel blockade by verapamil inhibits GMCs and diarrhea during small intestinal inflammation. Am. J. Physiol. 36: G785–G794, 1997.—The aim of this study was to investigate whether the blockade of L-type Ca\(^{2+}\) channels with verapamil suppresses giant migrating contractions (GMCs) and therefore diarrhea during small intestinal inflammation. Small intestinal inflammation was induced by infection with the nematode Trichinella spiralis. T. spiralis infection alone significantly increased the frequency of GMCs and decreased the frequency of phase III activity in the small intestine for 9 days. The increased frequency of GMCs was associated with diarrhea. Immunohistochemical staining with specific antibodies indicated that the number of neutrophils and mast cells increased significantly in the jejunal lamina propria during T. spiralis infection. Only the neutrophils increased significantly in the muscularis externa of the jejunum. Myeloperoxidase (MPO) activity increased significantly in the jejunal and ileal lamina propria. Daily verapamil administration during T. spiralis infection significantly reduced the frequency of GMCs and diarrhea but had no further significant effect on the already reduced frequency of phase III activity. Verapamil administration, however, did not reduce MPO activity or immunocyte infiltration in the jejunum or ileum. We conclude that blockade of L-type Ca\(^{2+}\) channels selectively reduces the frequency of GMCs and therefore diarrhea during small intestinal inflammation. The decreased frequency of GMCs is not secondary to a reduction in the inflammatory response.

The gastrointestinal smooth muscle cells generate several different types of contractions to perform the complex motility functions of mixing and propulsion (33). These include rhythmic phasic contractions, ultraphasic contractions, and an increase in tone (33). In the small intestine, the rhythmic phasic contractions, organized as migrating motor complexes (MMC), keep the upper gastrointestinal tract free of debris and limit bacterial overgrowth to the distal small intestine during the interdigestive state. In the postprandial state, the phasic contractions perform the mixing and orderly slow distal propulsion of the ingested meal. The precise physiological role of an increase in tone in circular muscle cells is not known. However, it is possible that the decrease in the diameter of the lumen due to a sustained increase in tone may enhance the effectiveness of phasic contractions in mixing and propulsion of chyme.

The ultraphasic contractions in the small intestine consist of giant migrating contractions (GMCs) and retrograde giant contractions (RGCs). In the normal state, GMCs occur once or twice a day in the terminal ileum and produce mass movements that may extend into the proximal colon (30, 32, 33). During intestinal inflammation, the frequency of GMCs is increased dramatically and the frequent mass movements in the fasting as well as the postprandial state result in diarrhea (1, 5, 13, 14, 27). Under certain conditions, GMCs may also produce abdominal pain (15, 30, 31). RGCs rapidly regurgitate the contents of the upper small intestine into the stomach in preparation for vomitus expulsion (18).

It is remarkable that the same circular smooth muscle cells can generate so many different types of contractions using a limited number of signal-transduction pathways. The force and duration of contractions generated by smooth muscle cells are related to the increase in [Ca\(^{2+}\)] (36). The amplitude of GMCs is two to three times greater and their duration four to six times longer than the corresponding parameters of phasic contractions in phase III activity (30). This suggests that a GMC may require a much greater increase in [Ca\(^{2+}\)] than a phasic contraction. Our hypothesis, therefore, is that partial or complete inhibition of one of the sources of Ca\(^{2+}\), such as Ca\(^{2+}\) influx, may affect the occurrence of GMCs more than it affects the occurrence of the phasic contractions. If so, Ca\(^{2+}\) channel blockers may reduce diarrhea associated with GMCs without having a major effect on the occurrence of phasic contractions and the motility functions that these contractions perform.
Ca\textsuperscript{2+} is a ubiquitous intracellular messenger. The blockade of Ca\textsuperscript{2+} channels may also affect the infiltration and activation of immunocytes. Given that Ca\textsuperscript{2+} channel blockade may selectively reduce the frequency of GMCs, we also sought to determine whether Ca\textsuperscript{2+} channel blockade is secondary to a reduction in the inflammatory response or due to a direct effect of the blockade of Ca\textsuperscript{2+} channels in the neuromuscular circuitry of the small intestine.

METHODS

Surgical Procedure

The experiments were performed on 25 dogs of either sex, each weighing 17–30 kg (23 ± 1 kg). The dogs were anesthetized with 30 mg/kg pentobarbital sodium (Abbott Laboratories, Chicago, IL). A midventral laparotomy was performed to gain access to the abdominal cavity. Eight strain-gauge transducers were attached to the seromuscular layer of the stomach and the small intestine. One transducer was located on the antrum, 5 cm proximal to the pylorus. The first transducer on the small intestine was located 15 cm distal to the pylorus, and the last transducer was located 15 cm proximal to the ileocolonic junction. The remaining transducers were distributed equal distances (57 ± 3 cm apart) between the first and the last transducer on the small intestine. All transducers were oriented to record circular muscle contractions. The lead wires of the transducers were brought out through a stainless steel cannula in the abdominal wall (29). The dogs were allowed to recover from surgery for 8–10 days.

Induction of Small Intestinal Inflammation by Trichinella Spiralis Infection

Male mice (CF-1) were used to maintain a stock infection of the nematode T. spiralis. Each mouse was fed 500 larvae orally. The larvae were allowed to mature in the mouse for at least 30 days before they were used to infect the dogs. The larvae were recovered from the mice by pepsin digestion of skeletal muscle, as described previously by Castro and Fairbain (3). Each dog was fed 2 × 10\textsuperscript{4} larvae/kg mixed with 50 g canned dog food.

Experimental Protocol

Each experiment was done after an overnight fast. Primary infection with the nematode T. spiralis occurs only once in each dog. Therefore, two separate groups of five dogs each were used. One group received T. spiralis alone, and the other received verapamil plus T. spiralis (Fig. 1). Preinfection control recordings were made for 1 wk from each dog.

Dogs in the first group (T. spiralis infection alone) were fed the larvae on Monday morning after one MMC was recorded in the duodenum to establish the interdigestive state. Daily 6-h recordings were made Monday to Friday for the next 2 wk (Fig. 1). The 2-wk recording period was chosen because the abnormal motility patterns and clinical symptoms of T. spiralis infection last for about 10 days (5, 6).

In the second group of dogs (T. spiralis infection plus verapamil), the effect of verapamil alone was first determined (Fig. 1). Control recordings were made for 1 wk. An intravenous infusion of 20 µg·kg\textsuperscript{-2}·min\textsuperscript{-1} verapamil was started 15 min after the occurrence of an MMC in the duodenum on Monday morning. The infusion and recordings were continued for 6 h. The dogs were orally fed a 120-mg tablet of verapamil at the end of the infusion and fed another tablet in the evening at 10 PM. This schedule was followed from Monday to Friday of the first week and on Monday and Tuesday of the second week. The dogs received 120-mg tablets of verapamil three times per day on Saturday and Sunday. The recordings of motor activity were made for 6 h daily.

Control recordings were made daily during the next week to establish that no effect of verapamil was left over (Fig. 1). On the following Monday, verapamil treatment was resumed (Fig. 1). The dogs were also infected with the nematode T. spiralis. Dogs were fed the larvae 1 h after the start of

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Fig. 1. Time chart of experiments in 2 groups of dogs. A: dogs infected with T. spiralis. B: dogs given verapamil. Daily 6-h recordings were made in all experiments.
verapamil infusion. Verapamil treatment continued until Tuesday of the following week. Daily 6-h recordings were made until the end of the second week.

Data Analysis

The recordings were made on a 12-channel Grass pen recorder. The lower and upper cutoff frequencies were set at direct current and 15 Hz, respectively. All data were analyzed visually. Each dog exhibited spontaneous MMC cycles consisting of four phases during the interdigestive state. Phase III activity was defined as a group of contractions occurring at maximum frequency; the group of contractions propagated as a whole in the caudad direction. The contractions in each group lasted for at least 6 min. Phase I had little or no contractile activity. Phase II had intermittent contractions and began when the average frequency of contractions over a 5-min period exceeded 10% of the maximum frequency during phase III activity. Phase IV was the period of transition from phase III to phase I and usually lasted 1–4 min. The interdigestive motor activity in the stomach also consisted of four phases as described above. Phase III activity in the stomach was defined as a series of clusters of one to seven contractions each, such that the quiescent period between successive clusters did not exceed 3 min. The maximum amplitude of at least one contraction in each cluster was greater than twice the mean amplitude of contractions in the preceding gastric phase II activity. The frequency of phase III activity in the small intestine and the stomach was determined by dividing the total number of phase III activities by the duration of the recording.

The GMC is a large-amplitude (2–3 times the amplitude of phase III contractions) and long-duration (4–6 times the mean duration of phase III contractions) contraction that rapidly propagates (~1 cm/s) in the caudad direction from the point of its origin. GMCs are ultrapropulsive and have been associated with diarrhea and abdominal cramping (5, 13, 14, 15, 27, 30, 31, 38). RGCs are also contractions of large amplitude (2–3 times the amplitude of phase III contractions) and long duration (2–3 times the duration of phase III contractions). RGCs usually originate in the mid-small intestine and propagate in the orad direction at a velocity of ~1 cm/s (18).

The mean maximum amplitude of contractions in phase III activity and the duration of phase III activity were quantified at three locations. The first transducer in the small intestine represented the duodenum, the second transducer represented the ileum, and the fourth transducer represented the jejunum. The mean maximum amplitude was determined by averaging the amplitudes of five maximum-amplitude contractions in phase III activity.

The data were analyzed in four periods: preinfection (5 days of recordings in the normal state), peak infection (days 2–5 postinfection), late infection (days 8 and 9 postinfection), and recovery (days 10–12 postinfection) (Fig. 1).

Measurement of Heart Rate and Blood Pressure

In four dogs, a Statham pressure transducer (model P23XL; Spectramed, Oxnard, CA) was implanted in the external carotid artery under general anesthesia (30 mg/kg Nembutal). Heart rate was monitored by electrocardiogram electrodes attached to the shaved skin.

Immunohistochemical Methods and Myeloperoxidase Assay

Immunohistochemical methods. Full-thickness intestinal tissues were obtained from five normal dogs with T. spiralis infection alone and five dogs treated with T. spiralis and verapamil as described above. The tissues were harvested under general pentobarbital sodium anesthesia (30 mg/kg iv). The tissues from the dogs with T. spiralis infection or T. spiralis infection plus verapamil were removed on day 4 postinfection, when the abnormal motility response was nearly maximum (5, 6).

Freshly removed segments of the jejunum (15–20 cm from the ligament of Treitz) and ileum (20–30 cm from the ileocecal junction) from anesthetized dogs were rinsed thoroughly with ice-cold phosphate-buffered saline (PBS), cut along the mesenteric border, pinned flat to their original dimension, and fixed in 4% paraformaldehyde solution at pH 7.4 for 24 h. The tissues were then rinsed in PBS three or four times. Immunohistochemical processing and analysis were done as described previously (14, 39). Three to four 10-µm cryosections were placed onto a gelatin slide. At least five slides from each dog were incubated with primary antibodies for 4 h at room temperature. Nonspecific antibodies were blocked with 5% goat serum for 30 min. The second antibody, rhodamine isothiocyanate, was visualized with the use of ultraviolet excitation at 520 nm after incubation at room temperature for 1 h. All immunocytes were counted blindly by one person (C. Singaram). Antibodies against B lymphocytes (CD3), T lymphocytes (CD4), interleukin-2 receptor (IL-2R) on lymphocytes, granulocytes (CD11b), activated dendritic cells (transfected follicular dendritic cell marker 1 [RFDC]), and the human leukocyte antigen DR-1 (HLADR)-positive cells were used. All immunocyte antibodies were purchased from Becton Dickinson (San Jose, CA) and used at 1:10 dilution. The number of immunocytes in the lamina propria and muscularis externa was counted per 10 villi or crypts under ×20 ultraviolet excitation of an Olympus BH2 microscope. Several lengths spanning 10 villi or crypts were counted from each section.

Mast cells. A newly developed and characterized antitrypsin polyclonal antibody raised in rabbit was used (kindly provided by Promega, Madison, WI) at 1:500 dilution (4). This primary antibody was incubated for 2 h at room temperature. Goat anti-chicken immunoglobulin G conjugated to tetramethylrhodamine was visualized with the use of rhodamine isothiocyanate, was visualized with the use of ultraviolet excitation at 520 nm after incubation at room temperature for 1 h. All immunocytes were counted blindly by one person (C. Singaram). Antibodies against B lymphocytes (CD3), T lymphocytes (CD4), interleukin-2 receptor (IL-2R) on lymphocytes, granulocytes (CD11b), activated dendritic cells (transfected follicular dendritic cell marker 1 [RFDC]), and the human leukocyte antigen DR-1 (HLADR)-positive cells were used. All immunocyte antibodies were purchased from Becton Dickinson (San Jose, CA) and used at 1:10 dilution. The number of immunocytes in the lamina propria and muscularis externa was counted per 10 villi or crypts under ×20 ultraviolet excitation of an Olympus BH2 microscope. Several lengths spanning 10 villi or crypts were counted from each section.

Mast cells. A newly developed and characterized antitrypsin polyclonal antibody raised in rabbit was used (kindly provided by Promega, Madison, WI) at 1:500 dilution (4). This primary antibody was incubated for 2 h at room temperature. Goat anti-chicken immunoglobulin G conjugated to tetramethylrhodamine was incubated for 2 h at room temperature and, after thorough rinsing with PBS, visualized at 573 nm. Quantitation of the number of positive cells was performed by the method described for other inflammatory cells. This antibody was previously found to be specific by neutralization experiments (using trypsin) and is known to stain both granulated and degranulated mast cells. Also, this antibody appears to stain both mucosal and vascular types of mast cells. We elected to use this antibody because we wanted to know the change in the total number of mast cells in our experimental condition.

Myeloperoxidase estimation. Myeloperoxidase (MPO) assay was performed by the method of Krawisz et al. (16). Briefly, tissue samples were weighed and homogenized with hexadecyltrimethylammonium bromide (HTAB) buffer (0.5% HTAB in 50 mM phosphate buffer, pH 6.0, 4°C). The homogenates were freeze-thawed three times and then centrifuged at 35,000 g for 30 min. The pellets were discarded, and the supernatants were assayed for soluble protein and for MPO activity. MPO activity was measured by adding 0.1 ml supernatant to 2.9 ml reaction buffer [50 mM phosphate buffer, pH 6.0, containing 0.167 mM o-dianisidine dihydrochloride (Sigma, St. Louis, MO) and 0.0005% hydrogen peroxide]. After 1 min, the change in absorbancy at 460 nm was measured. One unit of MPO activity was defined as that degrading 1 µmol of peroxide per minute at 25°C. The MPO activity was expressed per gram of protein. Soluble protein in the tissue supernatant was assayed using a protein assay kit.
(Pierce, Rockford, IL) as originally described by Lowry et al. (20).

Scoring of stools. The stool consistency was scored on a scale of 1 to 5: 1 = watery stools; 2 = soft unformed stools; 3 = normal formed stools; 4 = hard stools of small caliber; 5 = no stools. The frequency of stools could not be analyzed reliably because liquid stools tended to mix, and dogs may soil at multiple locations during the same defection. A score below 2.0 was defined as diarrhea and above 3.5 as constipation. The threshold for constipation and diarrhea is uneven around the normal stool score because total absence of stools with a score of 5 was a much less common occurrence than the incidence of liquid stools. Liquid stools and the minimum score of 1.0 can occur for several days in a row.

Statistical Analysis

All data are expressed as means ± SE. All normally distributed data were analyzed by analysis of variance. Multiple comparisons were done by Fisher’s least-square difference method. Student’s t-test was used for the comparison of two means. Nonparametric Student-Newman-Keuls test was used for data that did not pass the normality test. A P value of <0.05 was considered statistically significant. The study was approved by the Animal Studies Committee at the Zablocki Veterans Affairs Medical Center.

RESULTS

Clinical Symptoms

The mean stool scores in the preinfection state in the two groups of dogs, one receiving T. spiralis infection alone and the other receiving T. spiralis infection plus verapamil, were not different from each other (n = 5). Both scores indicated normal formed stools (Fig. 2). The stool score fell below 2.0 (P < 0.05) on days 2–5 and days 8 and 9 in dogs that received T. spiralis infection alone, indicating diarrhea. The stool score on days 10–12 in these dogs was still less than that in the normal state, but it was above the score of 2.0, indicating recovery.

The stool score in dogs infected with T. spiralis and given verapamil did not fall below 2.0 in any postinfection period (n = 5; Fig. 2). During each postinfection period, the stool score in verapamil-treated dogs was significantly closer to normal than in dogs not treated with verapamil (Fig. 2).

The daily caloric intake decreased significantly during days 2–5 in dogs treated with T. spiralis infection alone as well as in dogs treated with T. spiralis and verapamil (Fig. 3). There was no significant difference in daily caloric intake between verapamil-treated and non-verapamil-treated dogs during any postinfection period (n = 5; Fig. 3).

Verapamil infusion significantly decreased the mean systolic blood pressure by ~18% and increased the heart rate by ~40% (Table 1) at the end of the 6-h infusion period on day 1 (n = 4). Similar changes occurred during verapamil infusion in dogs infected with the nematode T. spiralis (Table 1). The blood pressure and heart rate on subsequent days were stable around the values seen at the end of the 6-h infusion period on day 1.

MPO Activity and Immunohistochemical Findings

Changes in immunocyte infiltration. The number of neutrophils and mast cells increased significantly in the lamina propria of the jejenum on day 4 of T. spiralis infection (Fig. 4; Table 2). This was also the time when the maximum effects of inflammation on motility patterns were seen (Fig. 4; Table 2). Ileal lamina propria exhibited a significant increase of T lymphocytes only (Table 2). Only neutrophils were found to increase in the jejunal muscularis externa of T. spiralis-treated dogs (Fig. 4; Table 2). The immunocyte infiltration in dogs treated with verapamil and T. spiralis was not different from those treated with T. spiralis alone, except for mast cells, which were significantly greater in the lamina propria of the ileum in dogs treated with verapamil.

Table 1. Effect of verapamil infusion on systolic blood pressure and heart rate

<table>
<thead>
<tr>
<th></th>
<th>Normal dogs</th>
<th>Dogs Treated with T. spiralis</th>
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<tr>
<td></td>
<td>Control</td>
<td>After 6-h infusion</td>
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<tr>
<td>Systolic blood pressure,</td>
<td>140 ± 10</td>
<td>116 ± 11*</td>
</tr>
<tr>
<td>mmHg</td>
<td></td>
<td></td>
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<tr>
<td>Heart rate, beats/min</td>
<td>91 ± 4</td>
<td>127 ± 8*</td>
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</tbody>
</table>

Data are means ± SE; n = 4. *P < 0.05 vs. control.
T. spiralis plus verapamil than in dogs treated with only T. spiralis infection (Table 2). All other immune cells, i.e., B lymphocytes, IL-2R, HLADR, and RFD1 cells, did not exhibit any significant change in T. spiralis or T. spiralis plus verapamil-treated dogs.

MPO activity. MPO activity increased significantly in the lamina propria of the jejunum and the ileum on day 4 of infection in both groups of dogs (Table 3). There was no significant difference between the dogs that received T. spiralis infection alone and the dogs that received T. spiralis infection plus verapamil. The MPO activity increased in the muscularis externa of the jejunum in dogs treated with verapamil and T. spiralis (Table 3).

Effect of T. Spiralis-Induced Inflammation on Gastrointestinal Motor Activity

Small intestinal inflammation due to T. spiralis infection significantly decreased the frequency of phase III activity in the stomach and the whole small intestine on days 2–5 and days 8 and 9 postinfection (n = 5; Fig. 5). The frequency of phase III activity in the small intestine, but not in the stomach, recovered on days 11 and 12 postinfection.

The frequency of GMCs increased significantly during intestinal inflammation induced by T. spiralis infection (Figs. 6A and 7). The dogs were often uncomfortable when GMCs occurred in the small intestine. The increase in the frequency of GMCs was significant on days 2–5 and days 8 and 9 postinfection.

Small intestinal inflammation increased the frequency of RGCs on days 2–5 only (Fig. 8).

Table 2. Effect of T. spiralis infection and verapamil treatment on infiltration of inflammatory cells in the small intestine

<table>
<thead>
<tr>
<th></th>
<th>Jejunum</th>
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<th>Ileum</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>T. spiralis infection alone</td>
<td>T. spiralis infection + verapamil</td>
<td></td>
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<tr>
<td>Neutrophils per 10 villi length</td>
<td></td>
<td></td>
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<tr>
<td>Lamina propria</td>
<td>7.5 ± 2.5</td>
<td>34.9 ± 6.4*</td>
<td>23.4 ± 4.6*</td>
<td>10.2 ± 1.0</td>
</tr>
<tr>
<td>Muscularis externa</td>
<td>4.3 ± 1.7</td>
<td>19.8 ± 12.1*</td>
<td>21.2 ± 3.6*</td>
<td>2.2 ± 0.9</td>
</tr>
<tr>
<td>Mast cells per 10 villi length</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamina propria</td>
<td>7.3 ± 1.9</td>
<td>16.0 ± 3.3*</td>
<td>29.1 ± 7.7*</td>
<td>7.5 ± 1.8</td>
</tr>
<tr>
<td>Muscularis externa</td>
<td>7.1 ± 2.9</td>
<td>7.1 ± 3.6</td>
<td>8.5 ± 1.9</td>
<td>8.8 ± 2.8</td>
</tr>
<tr>
<td>T lymphocytes per 10 villi length</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Lamina propria</td>
<td>1.8 ± 0.65</td>
<td>12.5 ± 4.8</td>
<td>4.3 ± 2.5</td>
<td>0.8 ± 0.24</td>
</tr>
<tr>
<td>Muscularis externa</td>
<td>3.9 ± 0.86</td>
<td>7.6 ± 2.7</td>
<td>2.0 ± 0.5</td>
<td>1.2 ± 0.4</td>
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<tr>
<td>Data are means ± SE; n = 5. *P &lt; 0.05 vs. control. †P &lt; 0.05 vs. T. spiralis infection alone.</td>
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stomach and the duodenum but not in the jejunum and the ileum (Table 4); 2) the mean duration of phase III activity in the stomach, duodenum, and ileum \( (n = 5) \); Table 3); 3) the maximum amplitude of phase III contractions in the jejunum and the ileum (Table 3); and 4) the percentage of phase I activity in the MMC cycle of the stomach, duodenum, jejunum, and ileum (Table 4).

Effect of Verapamil on Abnormal Motor Activity During Small Intestinal Inflammation Induced by \( T. \) Spiralis Infection

Although verapamil decreased the frequency of phase III activity in the small intestine in the normal state, it had no further effect on the already reduced frequency of phase III activity in the inflamed state \( (n = 5) \); Fig. 5). In the stomach, verapamil had no further effect on the already reduced frequency of phase III activity on days 2–5, but on days 8 and 9 the frequency of phase III activity in verapamil plus \( T. \) spiralis-treated dogs was less than that in dogs treated with \( T. \) spiralis alone (Fig. 5).

The frequency of GMCs in verapamil plus \( T. \) spiralis-treated dogs was significantly less than that in \( T. \) spiralis-treated dogs on days 2–5 and days 8 and 9 (Figs. 6B and 7). However, verapamil had no significant effect on the distance of origin of GMCs from the ileocolonic junction and the distance of their propagation \( (n = 5) \); Table 5).

Administration of verapamil during \( T. \) spiralis infection did not reverse the increase in the frequency of RGCs on days 2–5 postinfection (Fig. 8).

DISCUSSION

Our findings show that blockade of L-type \( \text{Ca}^{2+} \) channels by verapamil significantly inhibits the frequency of GMCs during small intestinal inflammation without having a significant further effect on the already reduced frequency of phase III activity. GMCs are large-amplitude and long-duration contractions that rapidly propel small intestinal, pancreatic, and biliary tract secretions in the fasting state and undigested food in the postprandial state into the colon to increase its osmotic load (6, 27, 30). During small intestinal inflammation, the GMCs originating in the small intestine also propagate into the colon to produce frequent colonic mass movements (14, 26, 35). Both factors contribute to diarrhea. The frequency of GMCs has also been reported to increase in human small intestinal inflammation during Salmonella and gram-negative bacilli infections (1, 13) and in animal models of inflammation including radiation enteritis (27), \( T. \) spiralis infection (5), and mucosal exposure to ethanol

Table 3. MPO activity during inflammation with and without verapamil treatment

<table>
<thead>
<tr>
<th>Lamina propria</th>
<th>Normal</th>
<th>( T. ) spiralis Infection</th>
<th>Verapamil + ( T. ) spiralis Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>13.4 ± 3.3</td>
<td>41.5 ± 8.5*</td>
<td>38.7 ± 7.3*</td>
</tr>
<tr>
<td>Ileum</td>
<td>15.9 ± 5.1</td>
<td>42.8 ± 11.1*</td>
<td>61.2 ± 8.44*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Muscularis externa</th>
<th>Normal</th>
<th>( T. ) spiralis Infection</th>
<th>Verapamil + ( T. ) spiralis Infection</th>
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<tr>
<td>Jejunum</td>
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<td>27.7 ± 6.5*</td>
</tr>
<tr>
<td>Ileum</td>
<td>10.8 ± 3.9</td>
<td>21.9 ± 6.6</td>
<td>16.3 ± 2.6</td>
</tr>
</tbody>
</table>

Data are means ± SE; \( n = 5 \). MPO, myeloperoxidase. * \( P < 0.05 \) vs. normal. † \( P < 0.05 \) vs. \( T. \) spiralis.
and acetic acid (14). Inflammation in all of these states produces diarrhea and abdominal cramping. The reduction in the frequency of GMCs by verapamil during small intestinal inflammation induced by \textit{T. spiralis} infection significantly reduced diarrhea. In contrast, verapamil had no significant effect on anorexia during \textit{T. spiralis} infection.

Although GMCs and RGCs are both giant contractions, there are significant differences in their characteristics. The RGCs propagate in the orad direction at a

Fig. 6. A: frequency of giant migrating contractions (GMCs), indicated by arrows, increased significantly during small intestinal inflammation induced by \textit{T. spiralis} infection. B: verapamil treatment decreased frequency of GMCs during small intestinal inflammation. ST, gastric strain-gauge transducer. SI, small intestinal strain-gauge transducer. Numbers after ST and SI indicate distance of transducer from pylorus. Tracings in A and B indicate gastrointestinal motor activity on day 4 after infection. Arrowheads show end of phase III activity.

Fig. 7. Small intestinal inflammation due to \textit{T. spiralis} infection significantly increased frequency of GMCs on days 2–5 and days 8 and 9. Verapamil treatment significantly decreased frequency of GMCs in both periods. Frequency of GMCs decreased to normal range in both groups of dogs on days 10–12. Solid bars, \textit{T. spiralis} alone; hatched bars, \textit{T. spiralis} plus verapamil. * \(P < 0.05\) vs. preinfection. \# \(P < 0.05\) vs. \textit{T. spiralis} infection.

Fig. 8. Small intestinal inflammation due to \textit{T. spiralis} infection significantly increased frequency of retrograde giant contractions (RGCs) on days 2–5. Solid bar, \textit{T. spiralis} alone; hatched bar, \textit{T. spiralis} plus verapamil. * \(P < 0.05\) vs. preinfection. Verapamil treatment had no significant effect on this increase.
velocity of ~10 cm/s (17), whereas GMCs propagate in the caudal direction at a velocity of ~1 cm/s. The RGCs generally originate at about the mid-small bowel, but the GMCs can originate anywhere in the small intestine (5, 27, 30). The duration of GMCs is about four to six times longer and that of RGCs about two to three times longer than the duration of phase III contractions. Our data suggest that the dependence of the two types of giant contractions on Ca²⁺ influx through L-type channels may be markedly different. Verapamil reduced the frequency of GMCs but not that of RGCs during small intestinal inflammation.

_T. spiralis_ infection in rats induces inflammation mainly in the proximal small intestine (2, 23). The MPO activity in _T. spiralis_-infected rats increases in the proximal jejunum but not in the ileum. Our data show that in dogs, small intestinal inflammation due to _T. spiralis_ infection may be more generalized. MPO activity increased in both the jejunum and the ileum. The stimulation of GMCs during inflammation is thought to be due to the modulation of the enteric neural and cellular mechanisms of control of contractions by the inflammatory response mediators. The increase of inflammatory cells throughout the small intestine is consistent with our finding that verapamil reduced the frequency of GMCs but not that of RGCs during small intestinal inflammation.

Although verapamil reduced the frequency of GMCs, it did not reduce the inflammatory response as measured by MPO activity or the infiltration of inflammatory cells. These data suggest that the reduction in the frequency of GMCs and accompanying diarrhea may be a direct effect of blocking Ca²⁺ channels in enteric neurons and smooth muscle cells rather than due to a reduction of the inflammatory response. The activation of nonexcitable immunocytes is also accomplished by an increase in [Ca²⁺]i that is partially due to Ca²⁺ influx. However, the precise nature and regulation of ion channels through which influx occurs in nonexcitable cells are not understood completely. There is strong evidence that Ca²⁺ influx in leukocytes or lymphocytes is not due to Ca²⁺ influx through voltage-gated ion channels (8, 10, 11). 1) The depolarization of leukocytes by high K⁺ that opens voltage-gated Ca²⁺ channels does not significantly increase [Ca²⁺]i. 2) Blockade of voltage-dependent Ca²⁺ channels does not block agonist-induced Ca²⁺ influx in lymphocytes. Ca²⁺ influx in the inflammatory cells is thought to occur through voltage-independent second messenger-operated channels (9).

These data support our finding that verapamil did not reduce the inflammatory response to suppress abnormal motility.

Ca²⁺ influx through L-type channels plays an important role in the occurrence of in vivo phasic contractions as well as GMCs (34, 37), in vitro phasic contractions of muscle strips (28), and increased tone in dissociated single smooth muscle cells (12, 17, 25, 41). The precise sources of Ca²⁺ utilized to stimulate GMCs are not understood completely. However, Ca²⁺ influx through VOCS has been reported to play an important role in the stimulation of GMCs (34). Spontaneous membrane depolarizations are obliterated during a GMC, probably due to a sustained depolarization of the cell membrane (30). This depolarization may open VOCS. Also, GMCs induced by close intra-arterial infusions of caffeine are blocked by verapamil (34). Thus, whereas Ca²⁺ influx through VOCS plays an important role in the stimulation of both phasic contractions and GMCs, our present data show that the sensitivity of this influx for the stimulation of the two types of contractions may be different. At the dose of verapamil used in our study, the frequency of GMCs was inhibited significantly, whereas there was no concurrent additional effect on

<table>
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<th>Table 5. Effect of verapamil treatment on the parameters of GMCs during small intestinal inflammation</th>
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<tr>
<td><strong>Distance of origin of GMCs from the ileocolonic junction, cm</strong></td>
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<tr>
<td>T. spiralis infection alone</td>
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<tr>
<td>T. spiralis infection + verapamil treatment</td>
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<tr>
<td><strong>Distance of propagation of GMCs, cm</strong></td>
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<tr>
<td>T. spiralis infection alone</td>
</tr>
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<td>T. spiralis infection + verapamil treatment</td>
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Data are means ± SE. GMCs, giant migrating contractions.
MMC cycling that had already been decreased by inflammation.

The inhibition of Ca\(^{2+}\) influx in the normal state produced a heterogeneous response in the gastrointestinal tract. Verapamil significantly decreased the frequency of phase III activity in the stomach and the duodenum but not in the jejunum and the ileum. On the other hand, the maximum amplitude of contractions in phase III activity decreased in the jejunum and the ileum but not in the stomach and the jejunum. These data suggest that the role of Ca\(^{2+}\) influx through VOCCs to stimulate phase III activity may differ in the upper and lower gastrointestinal tract. A difference in the sensitivity of L-type Ca\(^{2+}\) channels to stimulate tone in isolated segments of the duodenum and colon has also been reported previously (21).

We expected verapamil to increase the duration of phase I activity by inhibiting phase II contractions. However, the duration of phase I activity after verapamil decreased throughout the gastrointestinal tract. Lang et al. (19) reported previously that phase I activity is produced by an ascending inhibitory reflex stimulated by the distally propagating phase III activity. Verapamil significantly decreased the amplitude and duration of phase III contractions. The decrease in the duration of phase I activity may be due to the partial inhibition of phase III activity and hence the intensity of the ascending inhibitory reflex.

DePonti et al. (7) reported that intravenous infusion of ~17 μmol·kg\(^{-1}\)·min\(^{-1}\) verapamil for 3 h almost completely abolished MMC cycling. We found that this inhibition occurs only in the stomach and the duodenum, and not in the distal small intestine. In contrast, Thollander et al. (42) reported no significant effect of verapamil on MMC cycling in rats.

The dose of verapamil used in our study decreased the systolic blood pressure by ~20% and increased heart rate by ~40%. The dogs did not exhibit any apparent signs of discomfort or uneasiness during verapamil treatment.

In conclusion, in dogs, inflammation due to T. spiralis infection occurs throughout the small intestine. Verapamil, an L-type Ca\(^{2+}\) channel blocker, significantly inhibits GMCS during inflammation without a concurrent effect on the already decreased frequency of phase III activity. The selective inhibition of GMCS reduces diarrhea. The inhibition of GMCS by verapamil seems to be a direct effect of L-type Ca\(^{2+}\) channel blockade on smooth muscle cells and enteric neurons rather than due to a reduction of the inflammatory response. The L-type Ca\(^{2+}\) channel blockers may have a potential therapeutic role in minimizing diarrhea and abdominal cramping during small intestinal inflammation.

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