Disruption of Intestinal Motility by a Calcium Channel–Stimulating Autoantibody in Type 1 Diabetes

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Background & Aims: Autonomic neuropathy, including gastrointestinal dysfunction, is a common complication of type 1 diabetes; however, its cause is uncertain. This study aimed to test whether functional autoantibodies cause the gastrointestinal dysfunction. Methods: We used isolated mouse colon undergoing colonic migrating motor complex (MMC) activity to test for autoantibodies that disrupt colonic motility. Purified Immunoglobulin G (IgG) from patients with type 1 diabetes or from controls was tested either in vitro or after passive transfer. Pharmacological studies of the interaction between the IgG and L-type calcium channel activator (Bay K8644) and inhibitors (nicardipine and verapamil) were performed. The effect of IgG on nerve-evoked contraction of the vas deferens longitudinal smooth muscle was also assessed. Results: MMC activity was disrupted by IgG (0.2 mg/mL) from 8 of 16 patients with type 1 diabetes but not by control IgG. Passive transfer of diabetic IgG to mice also disrupted MMCs, showing access to the antigen in vivo. The acute effect of the autoantibody was mimicked by the dihydropyridine agonist, Bay K8644 (2–10 nmoL/L), and both Bay K8644 and the autoantibody competitively inhibited the effect on MMC contraction of the dihydropyridine antagonist, nicardipine. Diabetic IgG, but not control IgG, altered the nerve-evoked contractile activity of vas deferens smooth muscle effects mimicked by Bay K8644. Conclusions: A novel functional autoantibody that activates smooth muscle L-type calcium channels at the dihydropyridine binding site is produced specifically by patients with type 1 diabetes and may mediate gastrointestinal and autonomic dysfunction in these patients.

Type 1 diabetes is a chronic autoimmune disease of unknown etiology. It is characterized by destruction of β cells in the endocrine pancreas and, in as many as 50% of patients, by the development of autonomic neuropathy. Indeed, diabetes is the most common cause of autonomic failure, but the mechanisms causing autonomic dysfunction are poorly understood.

Autonomic neuropathy in diabetes is characterized by early and widespread changes to the function of organs innervated by small-diameter unmyelinated nerve fibers in enteric, sympathetic, and parasympathetic pathways. Its clinical manifestations include gastroparesis, nocturnal diarrhea, bladder atony, postural hypotension, persistent tachycardia, gustatory sweating, pupillary abnormalities, and erectile dysfunction. Once clinical manifestations of autonomic neuropathy occur, the estimated 5-year mortality is approximately 50%, worse than for any other complication of diabetes. Recent studies suggest that autonomic neuropathy is likely to underlie sudden death in type 1 diabetes. Nevertheless, despite its clinical importance, few studies have addressed the mechanisms underlying autonomic neuropathy in diabetes.

Although type 1 diabetes is an autoimmune disease, an autoimmune-mediated cause of autonomic neuropathy has received relatively limited attention. Lymphocytic infiltrates occur around unmyelinated nerves and autonomic ganglia in some diabetic patients; complement-fixing antibodies are detected to autonomic nerves in 10%–30% of patients with type 1 diabetes, and autoantibodies are produced to the neuronal and islet cell antigen, glutamic acid decarboxylase (GAD), in 75%–84% of patients with recent-onset type 1 diabetes. However, there is a poor correlation between the presence of anti-GAD autoantibodies and autonomic neuropathy. In addition, complement-fixing autoantibodies can be found in patients with type 2 diabetes, which is generally regarded as a metabolic and not autoimmune disease. GAD is an intracellular enzyme, and anti-GAD autoantibodies would therefore not be expected to have a functional effect on intact, living cells. Similarly, other autoantibodies such as anti-IA-2 (ICA512) autoantibodies bind to intracellular targets and are unlikely to play a role in disease pathogenesis, although they are useful disease markers. Identification of an autoantibody

Abbreviations used in this paper: DHP, dihydropyridine; GAD, glutamic acid decarboxylase; IA-2, islet antigen-2 (protein tyrosine phosphatase-like protein); MMC, migrating motor complex; VGCC, voltage-gated calcium channel.

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Table 1. Clinical and Serological Features of Patients With Type 1 Diabetes

<table>
<thead>
<tr>
<th>Px</th>
<th>Age (yr)/Sex</th>
<th>IA-2/GAD</th>
<th>Gastrointestinal Symptoms</th>
<th>Novel Diabetic Autoantibody</th>
<th>Heterochronic Contractions</th>
<th>Ectopic Contractions</th>
<th>Aborted MMCs</th>
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<tr>
<td>D1</td>
<td>56/M</td>
<td>+/-</td>
<td>Abdominal bloating, fullness</td>
<td>+</td>
<td>3.5</td>
<td>1.5</td>
<td>1.5</td>
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<td>+</td>
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<td>1</td>
<td>1</td>
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<td>-</td>
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<tr>
<td>D5</td>
<td>64/F</td>
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<td>5</td>
<td>0.5</td>
<td>1.5</td>
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<td>54/F</td>
<td>+/-</td>
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<td>4</td>
<td>1</td>
<td>0</td>
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<td>3</td>
<td>0.5</td>
<td>3</td>
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<td>+</td>
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<td>Abdominal bloating, fullness</td>
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<td>D14</td>
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<tr>
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<td>43/M</td>
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<td>Abdominal bloating, fullness</td>
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</table>

Abbreviations: +, absent; +, present.
*Each IgG sample was tested on 2 occasions; the average number of events occurring in a 30-minute period, 30 minutes after the addition of IgG is shown.

with a functional effect on intact cells leading to altered autonomic function would represent a major advance in understanding diabetic autonomic neuropathy and provide new approaches to the diagnosis and monitoring of disease and to treatment.

We have recently developed a new approach using physiological assays on intact tissues to identify novel autoantibodies that interfere with autonomic neurotransmission through a functional effect on neurotransmitter receptors or ion channels.12-14 We have now developed an assay using intact isolated colon undergoing spontaneous, neurally mediated migrating motor complex (MMC) activity to detect effects of autoantibodies on different activation and inactivation states of ion channels and receptors, which is not possible using more traditional approaches. Such a physiological approach has not previously been used to investigate whether functional autoantibodies are produced in type 1 diabetes that alter autonomic neurotransmission and contribute to the autonomic dysfunction.

Materials and Methods

Patients

Blood samples were obtained with informed consent from patients with type 1 diabetes (n = 16); type 2 diabetes (n = 5); other endocrine diseases, including Graves' disease (n = 2) and Addison's disease (n = 2); systemic rheumatic diseases including primary Sjögren's syndrome with autonomic dysfunction (n = 7); scleroderma with gastrointestinal dysfunction (n = 3); polymyositis (n = 1), and rheumatoid arthritis with secondary Sjögren's syndrome (n = 2); idiopathic dysautonomia (n = 2); Crohn's disease (n = 1); idiopathic abdominal pain (n = 2); and from healthy controls (n = 5). Clinical features of patients with type 1 diabetes appear in Table 1. Anti-GAD and anti-IA-2 antibodies were detected using internationally standardized techniques.15 HbA1C levels were determined at the time of serum collection by ion-exchange high-pressure liquid chromatography (HPLC) using a mono-S column.16 The study was approved by the Clinical Ethics Committee of Flinders Medical Centre.

Spontaneous Intestinal Motility In Vitro

MMC were studied using minor modifications of previously described methods.17 Procedures were approved by the Flinders University Animal Ethics Committee. Briefly, colons were excised from euthanized male BALB/c mice (25-35 g) and suspended in a 100-mL organ bath containing Krebs' solution (mmol/L): NaCl 118, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.5, NaHCO₃ 25.6, D-Glucose 11.0, and CaCl₂ 2.5) gassed with 95% O2/5% CO2, pH 7.4 at 37°C. The mechanical activity of the circular muscle was recorded by 4 force-displacement transducers (ADInstruments, Sydney, Australia) attached at 1.2-cm intervals to the mesenteric border of the colon under an initial tension of 6 mN. Transducer output was fed into a 4-channel amplifier (ADInstruments) and recorded via Chart 4.2 software and a PowerLab/8s data acquisition system (ADInstruments).

Experimental Protocol and Data Analysis

After a 60-minute equilibration period, 5 control MMC were recorded. Patient or control immunoglobulin (IgG, purified on a protein A-phenyl column (Sigma, St Louis, MO),18 was added to give a final concentration of 0.2 mg/mL. Preliminary studies indicated that this is the optimal
concentration of IgG in this assay and reflects the assay’s greater sensitivity compared with tissue strips, in which 0.6–1 mg/mL was optimal. After a 30-minute incubation, MMCs were recorded for a 30-minute period in the continued presence of IgG. Each IgG sample was tested on at least 2 occasions. Experiments using diabetic IgG were performed blind to whether the patient had type 1 or type 2 diabetes. In some experiments, Bay K8644 (1–10 nmol/L; Sigma), nicardipine (0.1–100 nmol/L; Sigma), or verapamil (1–300 nmol/L; Knoll, Lane Cove, New South Wales, Australia) were added instead of or in addition to patient IgG. MMC activity in untreated preparations was stable for the 4–5-hour duration of the experiments. Colonic MMCs were defined as previously described. For assessment of competitive or noncompetitive interactions of the IgG with drugs, the maximal amplitude of 4 consecutive MMCs in the presence of IgG before the addition of any drugs and after the addition of increasing concentrations of nicardipine or verapamil was measured using the peak parameters—peak amplitude function of the DataPad on Chart v4.2 software (ADInstruments) and the percentage inhibition of MMC amplitude were calculated. The resulting data were analyzed by nonlinear regression and sigmoidal concentration-response curves with unitary slope and baseline set at 0% using GraphPad Prism (version 3.0a for Macintosh, GraphPad Software, San Diego, CA). The concentration of nicardipine or verapamil required to cause 50% inhibition of MMC maximum contraction amplitude (inhibitory concentration [IC50]) was determined. Results are reported as the mean IC50 and the upper and lower 95% confidence intervals (CIs) and the R² value for the goodness of curve fit, where df is the degrees of freedom.

For passive transfer studies, mice were injected intraperitoneally with 10 mg/kg of normal serum and purified as described earlier, or on each of 2 consecutive days. The maximum injected volume was 500 µL. On the third day, mice were sacrificed and MMCs recorded as described previously.

Vas Deferens Assays

Male mice were euthanized and the vas deferens removed and mounted in 5-mL organ baths containing Krebs’ solution. Preparations were fixed at 1 and then connected by cotton to a force-displacement transducer (ADInstruments) at the other, under a resting tension of 10 mN. Hexamethonium (500 µM) and capsaicin (10 µM/L) were added to block nicotinic ganglionic transmission and desensitize sensory fibers respectively. Platinum ring electrodes were used to stimulate the sympathetic nerve fibers with trains (5) of 90 pulses at 10 Hz (pulse duration 0.3 ms, voltage 60V) at 90-second intervals and the resulting contractions recorded using Chart software. After 2 sets of 5 stimulation trains 30 minutes apart, patient IgG was added to give a final concentration of 0.6 mg/mL. After a 30-minute incubation period, preparations were again stimulated with 2 sets of 5 stimulation trains. In some experiments, Bay K8644 (1–10 nmol/L) was added instead of patient IgG. The maximum amplitude, the rate of increase of contraction, and full time of the first phase of contraction were measured between the 40th and 80th percentile using the peak parameters—peak amplitude function of the DataPad on Chart v4.2 software. Raw measurements before and after the addition of IgG or drug were compared by using paired t tests; a probability less than 0.05 was considered significant.

Results

IgG From Type 1 Diabetics Disrupts Colonic Migrating Motor Complex Activity

Spontaneous, regular MMCs were recorded as contractions migrating from proximal to distal colon at intervals of 4–6 minutes, separated by periods of quiescence, during which inhibitory motor activity occurs (Figure 1A). Activity was profoundly disrupted by purified IgG from 8 of 16 patients with type 1 diabetes (Figure 1B) but not by IgG from healthy controls (n = 5; Figure 1C). The typical pattern of MMCs was lost in the presence of diabetic IgG, and a number of new features became evident including heterochronic contractions (Figure 1D), which occurred at the proximal recording site out of phase with the MMCs, during the normally quiescent phase; abnormal, isolated, nonpropagating contractions occurred at sites other than the proximal lead (ectopic contractions; Figure 1E); and some MMCs aborted before propagating the entire length of the colon (Figure 1F). These effects became apparent within 10 minutes of addition of IgG and persisted for the duration of the experiment (4–5 hours). Because purified IgG was used, a complement-dependent mechanism for this effect can be ruled out; similarly, an effect of high glucose concentrations can be ruled out because the experiments were performed in Krebs’ solution with a normal glucose concentration (11 mmol/L). Disease specificity was confirmed using IgG from patients with other endocrine or autoimmune diseases or with gastrointestinal or autonomic disorders. Heterochronic contractions, ectopic contractions, and aborted MMCs were not observed in any assays testing IgG from patients with type 2 diabetes (n = 5), Graves’ disease (n = 2), Addison’s disease (n = 2), primary Sjögren’s syndrome with autonomic dysfunction (n = 7), scleroderma with gastrointestinal dysfunction (n = 3), rheumatoid arthritis with secondary Sjögren’s syndrome (n = 2), polymyositis (n = 1), idiopathic dysautonomia (n = 2), Crohn’s disease (n = 1), and idiopathic abdominal pain (n = 2). Levels of HbA1C did not differ significantly between type 1 patients with the autoantibody or type 1 patients lacking the autoantibody or type 2 diabetes (F[2,17] = 2.0; P > 0.05 by 1-way analysis of variance) discounting a nonspecific effect because of glycosylation of IgG. Thus, some patients with type 1...
diabetes produce a novel functional autoantibody that modifies autonomic neurotransmission in the colon.

Passive Transfer of the Diabetic Autoantibody Causes Intestinal Dysmotility

Passive transfer experiments are crucial to confirm a specific pathogenic role for postulated autoantibodies. Colons from mice injected with diabetic IgG that contains the novel diabetic autoantibody showed profound disruption of MMC activity (n = 2 different patient IgG samples; Figure 1H) in contrast to normal MMC activity recorded in colons from mice injected with diabetic IgG that lacked the novel autoantibody (n = 2 different patient IgG samples; Figure 1G) or with IgG from healthy individuals (n = 4 different IgG samples).

A Calcium Channel Agonist Mimics and an Antagonist Reverses the Effect of the Diabetic Autoantibody

Based on our experience using receptor and ion channel modulators in the MMC assay (unpublished data, Jackson MW, March 2003), we attempted to mimic the autoantibody effect pharmacologically using a drug that opens L-type voltage-gated calcium channels (VGCCs) in the colonic smooth muscle. These channels are sensitive to the dihydropyridine (DHP) class of calcium channel activators and inhibitors and are crucial for smooth muscle contraction. We found that 2-10 nmol/L of the DHP agonist, Bay K8644, produced effects similar to those of 0.2 mg/mL diabetic IgG on the MMC preparation (Figure 2A).

To test if a DHP inhibitor of L-type VGCCs reverses the effect of the IgG, 1 nmol/L nicardipine was added to the MMC preparations 30 minutes after the addition of patient IgG. Nicardipine reversed the disruptive effect of diabetic IgG from each of 4 patients tested (Figure 2B).

The Diabetic Autoantibody Binds to the DHP-Binding Site on L-Type VGCCs

We next investigated whether the functional autoantibody bound directly to the DHP binding site on L-type VGCCs by determining whether the diabetic autoantibody competitively altered the effect of the DHP antagonist, nicardipine, on MMCs. The DHP-binding site is fully conserved between human and mouse (Protein Blast; www.ncbi.nlm.nih.gov/BLAST/).

![Figure 1. Immunoglobulin G from type 1 diabetics disrupts migrating motor complex activity recorded in mouse isolated colon. (A) Normal pattern of migrating motor complex (MMC) activity recorded using 4 force transducers placed along the colon from proximal (top trace) to distal (bottom trace). The spontaneous contractile complex occurs every 4-6 minutes, separated by periods of quiescence. (B) The pattern of MMC activity is disrupted by IgG (0.2 mg/mL) from a patient with type 1 diabetes. (C) IgG from a healthy control has no effect on MMCs. (D) Heterochronic contractions (contractions occurring out of phase with the MMCs) are evoked only by diabetic IgG and are indicated by asterisks. (E) Diabetic IgG also evokes ectopic contractions (marked by asterisks) (i.e., isolated contractions occurring in a region other than the proximal colon). (F) After addition of diabetic IgG, some MMCs abort before propagating the entire length of the colon (aborted MMC marked by asterisks). (G) Injection of mice over 48 hours with 20 mg of diabetic IgG without the autoantibody does not alter MMC activity. (H) Severe disruption of MMC activity was observed in mice injected over 48 hours with 20 mg of diabetic IgG containing the autoantibody. Tracings represent 1500 seconds (A, C, E, F, G), 2000 seconds (B), and 1500 seconds (D, H).]

![Figure 2. The dihydropyridine class of drugs can mimic or reverse the effect of diabetic IgG on MMC activity. (A) The dihydropyridine agonist, Bay K8644 (10 nmol/L) mimics the effect of 0.2 mg/mL IgG from type 1 diabetics. Heterochronic contractions occur in the proximal colon and ectopic contractions in the distal colon (marked by asterisks) in the presence of Bay K8644 (right panel) but not in the untreated control colon (left panel). (B) The effect of diabetic IgG (left panel) is reversed by the addition of the dihydropyridine antagonist, nicardipine (1 nmol/L; right panel). Tracings represent 1500 seconds (A) and 1000 seconds (B).]
Figure 3. IgG from type 1 diabetics competitively inhibits the effect of nicardipine on MMCs. (A) Nicardipine causes a concentration-dependent inhibition of the amplitude of MMCs (solid curve, filled squares). The concentration-response curve is shifted to the right by the dihydropyridine agonist, Bay K8644 (2 nmol/L; broken curve, open triangles). (B) IgG from 3 patients with type 1 diabetes that disrupted MMCs caused a parallel shift to the right of the nicardipine concentration-response curve. The average for the 3 patients is shown (broken curve, open triangles) compared with the response in the presence of disease control IgG (solid line, filled squares). (C) IgG from 3 patients that did not disrupt MMC activity also had no effect on the nicardipine concentration-response curve. The effect of nicardipine in the presence of disease control IgG is shown by the solid curve (filled squares) and in the presence of autoantibody-negative diabetic IgG by the broken curve (open triangles). (D) Diabetic IgG interferes noncompetitively with the phenylalkylamine calcium channel blocker, verapamil. Verapamil causes a concentration-dependent inhibition of MMC amplitude (solid curve, filled squares); the maximum inhibition is decreased and IC50 increased in the presence of diabetic IgG (broken curve, open triangles), indicating a noncompetitive interaction.

In a normal mouse colon, nicardipine concentration dependently inhibits the amplitude of MMCs with an IC50 of 1.6 nmol/L (Figure 3A, solid line; Table 2). Pretreatment of the colon with 2 or 10 nmol/L Bay K8644 caused a significant parallel shift of the nicardipine concentration-response curve to the right (Figure 3A, broken line), confirming that the agonist and antagonist interact competitively at the DHP-binding site. The IC50s for nicardipine in the presence of 2 nmol/L and 10 nmol/L Bay K8644 were 2.9 nmol/L and 6.9 nmol/L, respectively (Table 2). IgG from each of 4 patients whose IgG was previously shown to disrupt MMCs caused a parallel shift to the right of the nicardipine concentration-response curve, resulting in a significant increase in the IC50. The magnitude of this increase varied from patient to patient (Table 2) and is a measure of the potency of that patient's autoantibody. Pooling the data for these patients showed an average increase in the IC50 to 3.3 nmol/L (95% CI 2.7–4.0 nmol/L; Figure 3B). By contrast, IgG from each of 4 patients that did not disrupt MMC activity did not have any significant effect on the nicardipine concentration-response curve or IC50 (Figure 3C; Table 2). Identical experiments performed using IgG from patients with type 2 diabetes, Sjögren's syndrome with autonomic dysfunction, or scleroderma with gastrointestinal dysfunction did not significantly increase the IC50 for nicardipine (Table 2). Thus, IgG from some type 1 diabetics contains an autoantibody that competitively inhibits DHP binding to L-type VGCCs.

Because the diabetic autoantibody appears to act specifically at the DHP-binding site, we tested whether the autoantibody would cause a noncompetitive inhibition of
Table 2. Nicardipine Inhibition of Migrating Motor Complexes After Acute Exposure to Diabetic or Control IgG In Vitro

<table>
<thead>
<tr>
<th>IgG/Drug</th>
<th>Nicardipine IC50 (nM)</th>
<th>95% CI for IC50</th>
<th>R² (df)</th>
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<tr>
<td>Untreated control</td>
<td>1.6</td>
<td>1.1–2.3</td>
<td>0.99 (25)</td>
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<td>Pooled disease controls</td>
<td>1.5</td>
<td>1.2–1.8</td>
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<td>Bay K 8644 (2 mM/L)</td>
<td>2.0</td>
<td>2.1–3.9</td>
<td>0.99 (14)</td>
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<td>Bay K 8644 (10 mM/L)</td>
<td>6.9</td>
<td>4.3–11.1</td>
<td>0.99 (5)</td>
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<tr>
<td>Type 1 diabetes: patients whose IgG disrupts MMC activity</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>4.8</td>
<td>3.3–6.2</td>
<td>0.94 (20)</td>
</tr>
<tr>
<td>D4</td>
<td>2.5</td>
<td>2.1–2.9</td>
<td>0.98 (17)</td>
</tr>
<tr>
<td>D6</td>
<td>3.2</td>
<td>2.0–6.2</td>
<td>0.99 (17)</td>
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<td>D18</td>
<td>2.7</td>
<td>2.1–3.4</td>
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<td>Type 1 diabetes: patients whose IgG do not disrupt MMC activity</td>
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<td>D17</td>
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<td>0.98 (20)</td>
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<td>0.57–1.4</td>
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<tr>
<td>D9</td>
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<td>0.93 (17)</td>
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<td>D14</td>
<td>1.2</td>
<td>0.94–1.6</td>
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<tr>
<td>S6</td>
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<td>Scl2</td>
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<td>1.1–2.8</td>
<td>0.90 (17)</td>
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<tr>
<td>DAS 2</td>
<td>1.4</td>
<td>0.72–2.66</td>
<td>0.99 (17)</td>
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*Pooled results of experiments using primary Sjögren’s syndrome and scleroderma IgG.

*Significantly different from IC50 in untreated control.

*Significantly different from IC50 in pooled disease control.

The action of the phenylalkylamine class antagonist, verapamil, that acts at a site distal from the DHP site on the L-type VGCC. In the presence of disease control IgG, the IC50 for verapamil was 36 nmol/L (95% CI 21–63 nmol/L; R²(390) = 0.79) (Figure 3D solid line) and the maximum inhibition of MMC amplitude was 95 ± 5%. Diabetic IgG noncompetitively inhibited the effect of verapamil; the maximum inhibitory effect was decreased to 85 ± 4%, and the IC50 increased to 50 nmol/L (95% CI 31–82 nmol/L; R²(223) = 0.89) (Figure 3D, broken line), confirming that the diabetic autoantibody acts at the DHP-binding site and modifies the effect of verapamil through an allosteric interaction.

The Diabetic Autoantibody Also Acts on L-Type VGCCs in the Vas Deferens

To show that the novel diabetic autoantibody acts on smooth muscle L-type VGCCs in another organ, we tested diabetic IgG for its effects on the contraction of the vas deferens longitudinal muscle, commonly used as an example of a sympathetically innervated organ. Electrical nerve stimulation at 10 Hz produced a characteristic rapid phasic contraction followed by a sustained, tonic contraction mediated by adenylate triphosphatase and noradrenaline, respectively. Bay K8644 (1–10 nmol/L) caused a decrease in the amplitude of the rapid phasic component of the contraction (79% ± 6.7% of control; P = 0.037), a decrease in the rate of contraction, as measured by the leading slope (72.3% ± 7.46% of control; P = 0.036), and an increase in the fall time of the rapid contraction (177.9% ± 58.0% of control; P = 0.021). Similarly, diabetic IgG (0.6 mg/mL) significantly decreased the maximum amplitude (64.6% ± 12.7% of control; P = 0.0043) and rate of contraction (60.3% ± 13.0% of control; P = 0.011) and increased the fall time of the rapid phase contraction (149.4% ± 33.5%; P = 0.028) (n = 3 different IgG samples tested in duplicate; Figure 4), confirming that the autoantibody effects are likely to be present in all smooth muscles. Multiple washes of the prepa-
tion did not wash out the effect of the diabetic IgG. There was no significant effect of IgG from diabetic patients without the calcium channel antibody (n = 3 different IgG samples tested in duplicate), of healthy control IgG (n = 3 different IgG samples tested in duplicate), or of disease control IgG on the vas deferens contraction (Figure 4).

**Discussion**

Diabetic autonomic neuropathy is assumed to be multifactorial, with hyperglycemia, microangiopathy, deficiency of growth factors, and autoimmune destruction contributing. However, autonomic neuropathy can occur in the face of excellent glycemic control, levels of glycated hemoglobin do not always correlate with neuropathic symptoms, reversal of chronic hyperglycemia does not reverse autonomic dysfunction, and nerve growth factor therapy has not been successful in treating human diabetic neuropathy.

Functional autoantibodies that act at ion channels or receptors and disrupt autonomic or cardiovascular function have been described in autoimmune diseases including Lambert-Eaton myasthenic syndrome (LEMS), scleroderma, and Sjögren’s syndrome. Autoantibodies specifically to L-type VGCCs have been proposed as pathogenic in amyotrophic lateral sclerosis and in Sjögren’s syndrome, although these results have not been confirmed. An IgM autoantibody in type 1 diabetes with indirect effects on L-type VGCCs in pancreatic β-cells acts via an unidentified soluble mediator to cause apoptosis.

We have identified a novel IgG autoantibody in patients with type 1 diabetes that acts as an agonist at L-type VGCCs in the smooth muscle of the colon, thereby modifying enteric neuroeffector transmission. The pharmacological data provide strong evidence that the autoantibody binds to the DHP-binding site present on the α1C subunit of smooth muscle L-type VGCCs and cannot be explained by autoantibodies acting at other sites such as voltage-gated potassium channels, as have been proposed in some other intestinal motor disorders. The autoantibody joins a small family of agonistic autoantibodies including anti-thyroid stimulating hormone (TSH) receptor autoantibodies in Graves’ disease and antimetabolotropic glutamate receptor-3 antibodies in Rasmussen’s encephalitis.

The autoantibody is detected on isolated whole colon, in which there is regular, ongoing activation of the L-type VGCCs. Because this ongoing activation of L-type VGCCs is not met in more conventional assays, we were unable to detect the autoantibody immunohistochemically on sections of frozen human pancreas and frozen or fixed mouse colon, by immunoblotting mouse colon lysates, by immunoprecipitation using lysates from cells expressing L-type VGCCs or by fluorescence-activated cell sorting analysis (data not shown). It is therefore not surprising that this autoantibody has remained undetected to date. This may reflect a fundamental difference between functional autoantibodies and marker autoantibodies to intracellular antigens. Functional autoantibodies in Graves’ disease, primary Sjögren’s syndrome, and scleroderma are similarly not detectable by standard immunohistochemical approaches. We suggest a parallel with Graves’ disease, in which functional autoantibodies directed against the TSH receptor are present in very low concentrations and are not detectable by immunoprecipitation and immunofluorescence techniques.

L-type VGCCs are characterized by a pore-forming α1 subunit containing the DHP-binding site that is absent in non-L-type VGCCs. The DHP site is completely conserved between α1C isoforms present in smooth muscle, cardiac muscle, and brain. The novel functional autoantibody caused significant disruption of gastrointestinal motility and altered vas deferens contraction in vitro, and the passive transfer experiments show the autoantibody has access to the channel in vivo. This suggests the autoantibody is likely to cause similar disruption of α1C subunit-dependent smooth muscle contraction in the gastrointestinal and genitourinary tracts and blood vessels in diabetic patients and contribute to the pathogenesis of symptoms related to these organs.

In tissues that have quiescent smooth muscle and largely inactive VGCCs at rest, the effect of the autoantibody would only be apparent during nerve stimulation, giving the impression of a neuropathy even though the defect is actually at the level of the smooth muscle. The novel diabetic autoantibody would also be expected to recognize the DHP of cardiac α1C subunits and may contribute to the characteristic cardiac changes in diabetic patients that are associated with sudden death. Interestingly, α1C subunits are present in β-cells of the pancreatic islets. This raises the intriguing possibility that the novel functional autoantibody arises as part of the immune response to the β-cells. Although the effects of the autoantibody on β-cells is unknown, short-term exposure may stimulate β-cell VGCCs, causing an increase in insulin release, whereas chronic exposure may lead to apoptosis or down-regulation of VGCCs and reduced insulin release.

The DHP, nicardipine, is used as an anti-hypertensive and would be expected to compete with the acute actions
of the anti-VGCC antibody. However, it is premature
to suggest DHP antagonists for amelioration of autonomic
neuropathy in type 1 patients with the autoantibody, as
the chronic effects of the autoantibody are currently
unknown. In other autoantibody-mediated disorders of
autonomic function, including LEMS, compensatory
changes have been shown in receptor or channel expres-
sion.25

The findings of this study provide a rationale for
investigation of the correlation between the presence of
the novel functional autoantibody and autonomic
dysfunction in a large cohort of type 1 diabetic patients.
Because the accessibility of the α1C subunit may differ
between patients and between organ systems, the clinical
manifestations of the autoantibody may be variable, as
occurs in other diseases with functional autoantibodies
such as LEMS26 or autoimmune autonomic neuropathy.39
A limitation of this study is that formal autonomic
function testing has not yet been performed on the
patients; nevertheless, each of the 7 patients with type 1
diabetes who reported symptomatic gastrointestinal
dysfunction, of which reported abdominal bloating and
fullness, predictors of gastroparesis,40 had the novel func-
tional autoantibody (Table 2). By contrast, patients with
other endocrine or autoimmune diseases and gastroin-
testinal dysfunction did not have the autoantibody. Seven
of the 11 patients in this study with a large-fiber neurop-
athy had the novel functional autoantibody; whether the
autoantibody plays a pathogenic role in this type of
neuropathy awaits further study. However, studies in
animal models of type 1 diabetes indicate that serum
from diabetic Bio-Breeding/Wistar rats with large fiber
neuropathy enhances L- and T-type calcium currents in
primary sensory neurons.61

In conclusion, unique IgG autoantibodies are present
in the blood of a subset of patients with type 1 diabetes.
These autoantibodies cause profound disruption of gas-
троintestinal function by stimulating L-type VGCCs at
the DHP-binding site, and their effects are reversed by
L-type calcium channel blockers. Because L-type VGCCs
are present in smooth muscles throughout the body and
in neurons, these newly discovered diabetic autoantibod-
ies may also contribute to symptoms affecting other
peripheral organs and sensory fibers. We believe that
these findings represent a major conceptual advance in
understanding antibody-mediated autonomic dysfunc-
tion in type 1 diabetes.

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