Patients with long-standing diabetes commonly suffer from gastric neuromuscular dysfunction (gastropathy) causing symptoms ranging from postprandial bloating to recurrent vomiting. Autonomic neuropathy is generally believed to be responsible for diabetic gastropathy and the underlying impairments in gastric emptying (gastroparesis) and receptive relaxation, but the specific mechanisms have not been elucidated. Recently, it has been recognized that interstitial cells of Cajal generate electrical pacemaker activity and mediate motor neurotransmission in the stomach. Loss or defects in interstitial cells could contribute to the development of diabetic gastropathy. Gastric motility was characterized in spontaneously diabetic NOD/LtJ mice by measuring gastric emptying and by monitoring spontaneous and induced electrical activity in circular smooth muscle cells. Interstitial cells of Cajal were studied by Kit immunofluorescence and transmission electron microscopy. Diabetic mice developed delayed gastric emptying, impaired electrical pacemaking, and reduced motor neurotransmission. Interstitial cells of Cajal were greatly reduced in the distal stomach, and the normally close associations between these cells and enteric nerve terminals were infrequent. Our observations suggest that damage to interstitial cells of Cajal may play a key role in the pathogenesis of diabetic gastropathy.


Gastric neuromuscular dysfunction occurs in up to 30–50% of patients after 10 years of type 1 or type 2 diabetes (1–4). Symptoms of diabetic gastropathy can range from mild dyspepsia to recurrent vomiting and abdominal pain (1,3) and may progress to irreversible end-stage gastric failure known as gastroparesis (1–4). Gastroparesis seriously affects quality of life. There is deterioration in glycemic control (1,3) and incapacitating symptoms such as malnutrition, water and electrolyte imbalance, and aspiration may occur (1,5). Diabetic gastropathy and gastroparesis, including impaired fundic and pyloric relaxation (1) and impaired electrical pacemaking (1,2,6–8), are generally considered to be the result of systemic and/or enteric neuropathies (1–3,5,9,10); however, the pathophysiology of these disorders is not fully understood (1–3,5).

Numerous physiological studies have demonstrated that electrical pacemaking (11,12) and motor neurotransmission in the stomach (13–15) depend on the function of interstitial cells of Cajal (ICC) (16,17). Multipolar ICC, which form two-dimensional networks in the myenteric region of the gastric corpus and antrum (IC-MY), have been identified as the source of electrical slow waves that underlie the phasic contractions of the gastric musculature (11,12). We have recently demonstrated that the loss of gastric IC-MY, precipitated by inhibition of signaling via the receptor tyrosine kinase, Kit, results in the loss of electrical slow waves and eliminates the ability of the musculature to generate slow waves in response to depolarizing stimuli (12). Thus, functional ICC are critical for the production and maintenance of normal slow-wave activity and gastric motility. Depletion of these cells caused by pathological conditions would lead to impaired motility.

ICC do not require inputs from the autonomic or enteric nervous systems for development or function as pacemakers (18). Nevertheless, ICC are usually closely associated with neural elements of the gut wall. Spindle-shaped ICC running parallel to smooth muscle cells in the gastric fundus, corpus, antrum, as well as in the lower esophageal and pyloric sphincters (intramuscular ICC [IC-IM]), mediate excitatory and inhibitory inputs to the musculature from enteric motor neurons (13–15). As demonstrated by studies of W/Wmice, which lack gastric IC-IM, loss of these cells seriously impairs neural control of motility (13–15).

It follows from the aforementioned studies that ICC play a major role in both myogenic and neurogenic aspects of gastric motility. Significant motor abnormalities resembling those occurring in diabetic gastropathy and gastroparesis would arise if these cells were damaged or lost because of diabetes. Therefore, we have explored the hypothesis that depletion and/or remodeling of gastric ICC networks may contribute to gastropathy and gastroparesis in spontaneously diabetic mice. Our observations suggest the novel concept that ICC play a key role in the pathogenesis of diabetic gastropathy. Recognition of the role of ICC in this widespread complication of diabetes may provide a basis for more effective treatments.

RESEARCH DESIGN AND METHODS

NOD/LtJ mice of either sex were obtained from breeder pairs purchased from the Jackson Laboratory (Bar Harbor, ME). This mouse strain is susceptible to the spontaneous development of T-cell–mediated autoimmune insulitis and thus represents a model of human type 1 diabetes (19). BALB/c mice of either sex were obtained from breeder pairs purchased from Harlan Sprague-Daw-
le (Indianapolis, IN). The animals were maintained and experiments performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animals, and protocols were approved by the Institutional Animal Use and Care Committee at the University of Nevada.

NOD mice were screened for diabetes biweekly after reaching 8 weeks of age. Glyceroluria was assessed semi-quantitatively using the Chemstrip uGK urine glucose test (Boehringer Mannheim, Indianapolis, IN), which is based on the glucose oxidase/peroxidase method. Animals with detectable glyceroluria were considered diabetic. In cases of ambiguous or low levels of glyceroluria, diagnosis of diabetes was further tested by measuring glucose in a drop of whole blood obtained by puncturing a tail vessel. Blood glucose measurements were performed with an Accu-Chek Complete monitor (Boehringer Mannheim), which uses biamperometry of reduced hexacyanoferate (II) generated during the conversion of glucose to gluconolactone by glucose dehydrogenase. This method was validated by calibrating the monitor with glucose solutions: An eight-point assay covering glucose concentrations ranging from 50 to 600 mg/dl (2.77–33.30 mmol/l) resulted in an excellent linear correlation between the expected and measured levels (y = –13 + 1.024x, r = 0.995, r² = 0.990, P < 0.001).

Glucose concentrations exceeding 600 mg/dl (33.30 mmol/l) were above the upper limit of detectability. In these tests, animals were considered diabetic if blood glucose levels exceeded 250 mg/dl (13.87 mmol/l) and were used as controls if blood glucose levels remained <140 mg/dl (7.77 mmol/l). For the purpose of numerical analysis, blood glucose concentrations above the upper limit of detectability were assigned the value of the maximum detectable level (600 mg/dl, 33.30 mmol/l). In our colony, 64% of the females and 56% of the males developed diabetes at a median age of 6 months (range 4–11) (n = 70). Animals were used when signs of gastrointestinal (distended abdomen), neurological (impaired locomotion), and ophthalmologic (impaired vision) complications became evident by inspection (between 1.5 and 3 months, average 58 ± 5 days, n = 24) after the diagnosis of diabetes. Nondiabetic age-matched littermates (n = 37) were used as controls. Mean blood glucose concentrations, measured in whole-trunk blood obtained when they were killed, were 457 ± 29 mg/dl (25.36 ± 1.61 mmol/l) in the diabetic group and 104 ± 4 mg/dl (5.77 ± 0.22 mmol/l) in the nondiabetic group of NOD mice. Animals were killed under CO₂ anesthesia by cervical dislocation followed by decapitation. The fundus and the entire gastric corpus and antrum were separated and the mucosa was removed by sharp dissection. The junction between fundus and corpus was based on the clear mucosal demarcation between these regions in the mouse. Circular muscle cells were impaled with glass micro-electrodes to measure transmembrane potential, as previously described (12–15,18,20). The gastric corpus and antrum were divided into regions along their axes and each region was electrically mapped by multiple intracellular impalements (12). In the fundus, parallel platinum electrodes were used for electrical field stimulation (EFS) (12–15,18,20).

ICC were identified in acetone-fixed tissues (4°C, 10 min) with antibodies to the receptor tyrosine kinase, Kit (monoclonal rat anti–mouse IgG; Gibco, Gaithersburg, MD) (5 × 10⁻⁶ g/ml, 48 h at 4°C), as previously described (12–15,18,20). Immunoreactivity was detected using secondary antibody conjugated with Alexa Fluor 488 (anti–rat IgG; Molecular Probes, Eugene, OR) (12–15,18,20). The gastric corpus and antrum was based on the clear mucosal demarcation between these regions and the mucosa was removed by sharp dissection. The junction between fundus and corpus was based on the clear mucosal demarcation between these regions in the mouse. Circular muscle cells were impaled with glass micro-electrodes to measure transmembrane potential, as previously described (12–15,18,20). The gastric corpus and antrum were divided into regions along their axes and each region was electrically mapped by multiple intracellular impalements (12). In the fundus, parallel platinum electrodes were used for electrical field stimulation (EFS) (12–15,18,20).

Transmission electron microscopy was performed on ultrathin sections stained with uranyl acetate and lead citrate, as previously described (13,20). Sections were examined with a Philips CM10 electron microscope. ICC were identified by ultrastructural criteria (Fig. 3). For numerical analysis, nuclei of all cell types (452 ± 59 per animal) were counted across the entire thickness of the antral musculature. The number of ICC in the myenteric region (IC-MY) was expressed as the percent of total cell count. The proportion of IC-M cells in the circular and longitudinal muscles was expressed as the percent of smooth muscle cells in the corresponding muscle layer.

Gastric emptying was measured by a method modified from a previously described technique (21,22): After an overnight fast with free access to water, a 0.25 ml meal, consisting of one part powdered mouse diet suspended in two parts of water (wt/wt), was given by gavage using an 18-gauge animal feeding tube (Fisher Scientific, Pittsburgh, PA) under brief CO₂ anesthesia. After the meal, mice were returned to their home cages without food or water and killed after 3 h. The stomachs were removed after clamping the esophagus and the pylorus. Gastric contents were recovered and dried until weight constancy. Gastric emptying was expressed as the percent of solids emptied. In serious gastroparesis, emptying of the stomach may not be complete by the end of a 14- to 15-h fast. Such residual food would result in a spuriously reduced in the measured gastric emptying values. Therefore, we verified in three long-term diabetic mice that no food remained in their stomachs after an overnight (>24 h) fast.

RESULTS

The use of NOD mice as a model of diabetic gastroparesis was first characterized in terms of overall gastric motility. Gastric emptying was measured in diabetic and nondiabetic mice (Fig. 1A). Preliminary time-course experiments (four time points) performed in a population of BALB/c mice demonstrated that gastric emptying of the meal was complete in 3 h (96 ± 1% n = 4) (Fig. 1A, left panel). Similar results were found in two nondiabetic (94 and 98%) and two short-term diabetic (≤2 weeks) NOD mice (89 and 97%). These four animals served as a control NOD group (94 ± 2%). Gastric emptying was significantly less during the 3-h test period in NOD mice that had been diabetic for >1.5 months i.e., 45 ± 16% at 3 h, n = 4, P < 0.02) (Fig. 1A, right panel). These findings document gastroparesis in NOD diabetic mice.

The stomach is normally paced by electrical slow waves generated in the corpus (1,16). Gastric dysrhythmias (i.e., deviations from the normal slow-wave rhythm) interfere with peristaltic contractions, which facilitate emptying of food from the stomach and contribute to gastroparesis (1,2,6–8). Abnormal slow-wave activity has also been reported in streptozotocin-diabetic rats (23). We characterized spontaneous electrical activity of the gastric corpus and antrum in nondiabetic and diabetic NOD animals with intracellular electrophysiological techniques (12) (Fig. 1B). Resting membrane potentials (RMPs) of nondiabetic stomachs averaged 52 ± 1 mV (n = 25 cells in 7 tissues) and 56 ± 2 mV (n = 27 cells in 9 tissues) in circular muscle cells of the corpus and antrum, respectively. All cells were electrically active and displayed slow waves with amplitudes of 10 ± 1 mV (same in corpus and antrum) and frequency of 8.6 ± 0.5 cpm (corpus) and 8.0 ± 0.9 cpm (antrum) (Fig. 1B). The electrical activity of the oral corpus of diabetic animals was not different from that of the controls (n = 17 cells in 7 tissues). However, antral cells of diabetic mice were depolarized and most cells were electrically quiescent (RMP = 49 ± 2 mV, n = 25 cells in 6 tissues, P < 0.03 vs. nondiabetic) (Fig. 1B). The proportion of spontaneously active cells decreased from the distal corpus (78%) toward the pylorus. Only 19% of cells in the distal antrum displayed slow-wave activity (Fig. 1C). Both the frequency (5.3 ± 1.2 cpm) and amplitude (7.6 ± 1.1 mV) of slow waves recorded from the remaining active cells in the antrum (n = 6 cells in 4 tissues) were reduced below control levels, although the changes did not reach statistical significance. The impairments in electrical pacemaker activity in NOD diabetic mice are very similar to those described in streptozotocin-diabetic rats (23) and are likely to contribute to gastroparesis in these animals.

In diabetic patients, the fundus does not relax properly in response to distension (1,2,5,24,25). This reduces the retention of gastric contents in the proximal stomach and may contribute to dyspepsia in diabetic gastropathy (1,2,5,24,26,27). Impaired receptive relaxation could be reduced input from enteric neurons, and reduced inhibitory junction potentials (IJPs) have been reported in fundus muscles of strep-
tozotocin-diabetic rats (23). Therefore, we characterized responses to the EFS of fundus muscles from diabetic NOD mice and nondiabetic littermates. RMPs of control circular muscle cells were \(-43 \pm 2 \text{ mV (} n = 18 \text{ cells in 9 tissues)}\), and all cells were electrically quiescent (Fig. 1B–D). EFS evoked excitatory junction potentials (EJPs) followed by IJPs (Fig. 1D). EJP amplitudes averaged \(4.9 \pm 0.9, 5.7 \pm 0.9, \text{ and } 8.3 \pm 0.9 \text{ mV in nondiabetic tissues in response to } 5, 10, \text{ and } 20 \text{ Hz stimuli, respectively. IJP Ps averaged } 5.6 \pm 0.8, 6.9 \pm 0.8, \text{ and } 8.4 \pm 1.3 \text{ mV in these experiments. In four of five diabetic muscles, the amplitudes of EJPs and IJPs were dramatically reduced (Fig. 1D): EJP amplitudes averaged } 1.9 \pm 0.8 (P < 0.05\), and IJP Ps averaged \(1.9 \pm 0.8 (P < 0.05\).
Kit-like immunoreactivity (Kit-LI) was observed in multipolar cells with fusiform cell bodies in the myenteric region of the corpus and antrum (IC-MY) and in spindle-shaped cells running parallel to the muscle fibers in the circular and longitudinal muscle layers (IC-IM) in the fundus, corpus, and antrum of NOD mice with immunohistochemical studies. ICC were identified with antibodies to the receptor tyrosine kinase, Kit (12–18,20,28–30).

Surprisingly, spontaneous electrical rhythmicity developed in fundus muscles of diabetic animals, a region of the stomach that is normally quiescent (1,13,15). Electrical oscillations were recorded in 9 of 28 impalements in 2 of the 5 diabetic fundus muscles (i.e., amplitude 3.1 ± 0.4 mV, frequency 7.7 ± 1.4 cpm) (Fig. 1B). The frequency of oscillations in diabetic fundus muscles was similar to slow waves recorded from the corpus of the same animals. RMPs of active fundus cells were not different from control cells or quiescent diabetic cells.

Delayed gastric emptying, loss of electrical rhythmicity, and reduced response to enteric nerve stimulation demonstrate that NOD diabetic mice develop gastric motility disorders that resemble those associated with diabetic gastropathy and gastroparesis (1–8,23–26). The losses of function observed in NOD diabetic stomachs could be due to disruptions in networks of ICC, which mediate electrical pacemaking and postjunctional neural responses in the stomach (11–17). Defects in ICC networks have been noted in other disorders of gastric motility (28). Hence, we studied the distribution and morphology of ICC in NOD mice with immunohistochemistry and transmission electron microscopy. Immunohistochemical studies, ICC were identified with antibodies to the receptor tyrosine kinase, Kit (12–18,20,28–30).

Kit-like immunoreactivity (Kit-LI) was observed in multipolar cells with fusiform cell bodies in the myenteric region of the corpus and antrum (IC-MY) and in spindle-shaped cells running parallel to the muscle fibers in the circular and longitudinal muscle layers (IC-IM) in the fundus, corpus, and antrum of nondiabetic NOD mice (n = 8) (Fig. 2A, C, E). The distribution of ICC was indistinguishable from that of wild-type animals (12,13,15,18). The ultrastructure of gastric IC-MY and IC-IM was examined by transmission electron microscopy, and these cells were identified by commonly recognized features (13,15–17,20,31,32) (Fig. 3A and C). IC-MY and IC-IM were usually closely associated with enteric ganglia or nerve fibers within the muscle layers (13,15–17,20,28,30–32) (Fig. 3A and C).

ICC were reduced in antral muscles of 11 of the 12 diabetic mice examined. The reduction in ICC was not homogeneous. Patches up to several square millimeters (which encompassed the distal half of the stomach in four animals) were found in which ICC were greatly reduced in number. Loss of ICC was greatest in the distal antrum, and less reduction was observed in the corpus. The distal corpus was affected in four mice, and ICC in the orad half of the corpus appeared normal in all animals. These observations closely parallel our electrophysiological findings that many cells in the antrum, but none in the orad corpus, were electrically quiescent in diabetic animals (Fig. 1B and C). Closer examination of the affected areas revealed that the reduction in Kit-LI was due to a substantial decrease in ICC number (Fig. 2B, D and F). IC-MY were completely missing in many areas in nine tissues and severely reduced in another two. A prominent reduction of IC-IM was observed in the circular muscle of seven and in the longitudinal muscles of six diabetic mice. In the four animals displaying reduced Kit-LI in the orad half of the corpus, only IC-MY were decreased in number. The remaining cells often had tortuous and irregularly branching processes (Fig. 2F).

Examination of the distal antrums of three diabetic animals with transmission electron microscopy confirmed that the reduction in Kit-LI was caused by a reduction in ICC. In diabetic animals, enteric nerve fibers and ganglia were frequently observed without associated ICC (Fig. 3B). ICC in the distal antrum of three control and three diabetic mice were counted in electron micrographs. In controls, IC-MY represented 11.2 ± 0.6% of cells across the entire musculature, whereas IC-IM in the circular and longitudinal muscles represented 15.7 ± 1.6% and 17.0 ± 1.8% of smooth muscle cells, respectively. In the diabetic animals, IC-MY were reduced to 5.5 ± 0.4% (P < 0.005 vs. nondiabetic) and IC-IM in circular and longitudinal muscles were decreased to 7.7 ± 2.1% (P < 0.05) and 7.2 ± 2.6% (P < 0.05), respectively. Remaining ICC in diabetic tissues (Fig. 3D) had obviously reduced perinuclear cytoplasm without notable changes in the density of intracellular organelles. Processes were atrophied and displayed increased branching. IC-MY were frequently separated from enteric ganglia by wide extracellular spaces. No signs of apoptosis or necrosis were observed, so the mechanisms responsible for the morphological changes and loss of ICC remain unclear.

The gastric fundus was examined with Kit immunohistochemistry in 10 diabetic NOD mice and 8 nondiabetic littermates. Only IC-IM running parallel to circular and longitudinal muscle cells were observed in seven nondiabetic mice, as previously described (13,15) (Fig. 4A and C). In one nondiabetic animal, a loose network of multipolar ICC was observed in the myenteric region near the border of the corpus. With fluorescence microscopy, the distribution of IC-IM did not appear altered in diabetic animals. However, in 50% of the diabetic mice, extensive networks of multipolar (i.e., IC-MY–like) cells were found in the fundus. These cells were continuous with IC-MY networks of the corpus but extended several millimeters into the fundus (Fig. 4B and D). Transmission electron microscopy showed that IC-1M, although not reduced in number, lacked the typical close associations with nerve terminals that have been described previously and suggested to be critical for enteric motor neurotransmission in the fundus (13,15) (Fig. 5).

There were no detectable ultrastructural defects in enteric neurons or in smooth muscle cells in the antrum or fundus of diabetic mice. Nevertheless, we cannot exclude the possibility that reductions in neurotransmitter release, alterations in excitability mechanisms, or intracellular signal transduction in smooth muscle cells may also accompany diabetic gastroparesis (9,10,33).

**DISCUSSION**

Despite reports of diabetic gastroparesis without autonomic neuropathy (1,34,35), this disease, and, particularly, impaired electrical pacemaking (1,2,6–8), are usually considered to be the result of systemic and/or enteric neuropathies (1–3,5,9,10). Recent studies in spontaneously diabetic BioBreeding/Worcester rats (9) and in streptozotocin-diabetic rats (10) have demonstrated decreased nitric oxide synthase expression and reduced nitricergic motor inputs to the stomach, as well as impaired intracellular signaling in response to excitatory neurotransmitters in gastric smooth muscle (33). Clearly, these defects may contribute to the many abnormal features of dia-
betic gastropathy. However, it is unclear how impaired neural inputs (especially impaired inhibitory inputs) or impaired smooth muscle response to cholinergic stimulation could result in the loss of slow-wave activity, which has been observed in both humans and animals with diabetes.

Physiological studies have demonstrated that electrical pacemaking depends on the function of ICC (11,12), and these cells do not require neural inputs from the autonomic or enteric nervous system for development or function as pacemakers (18). In the present study, we explored the hypothesis that depletion and/or remodeling of gastric ICC networks may contribute to the pathogenesis of diabetic gastropathy and gastroparesis in a murine model of diabetes. These studies were performed using the NOD/LtJ mouse strain, a widely used model of human type 1 diabetes (19). We observed a massive depletion of IC-MY (gastric pacemaker ICC) in the stomachs of diabetic NOD mice. Normal distributions of IC-MY networks were observed in age-matched nondiabetic NOD littermates. The decrease in Kit-LI in diabetic mice was most pronounced in the distal antrum, whereas little or no change was found in the corpus. Loss of pacemaker cells in the distal antrum was also verified by quantitative ultrastructural analy-
sis. The remaining ICC showed atrophic changes, and their processes displayed increased branching. We observed no signs of apoptosis or necrosis, so the mechanisms responsible for the morphological changes and loss of ICC are unclear. Loss of IC-MY was associated with a gradient in slow-wave activity: Slow-wave activity was nearly normal in the corpus and significantly abnormal in the antrum, including large areas of electrical quiescence. Previously, we have shown that selective ablation of gastric ICC, induced by inhibition of Kit signaling in these cells, results in loss of slow waves and eliminates the ability of the musculature to generate slow waves in response to stimuli (12). Moreover, similar to small intestinal ICC (16,17), gastric ICC are capable of generating rhythmic slow waves (T.W. Kim, S.D. Koh, K.M.S., unpublished data). Therefore, the decrease in IC-MY in diabetic NOD mice is likely to be the fundamental cause for the loss of slow-wave activity.

A second class of ICC, IC-IM, were also greatly reduced in the antrum. IC-IM are known to mediate excitatory and inhibitory inputs from motor neurons in the stomach (13–15). Although cells with Kit-LI were not obviously reduced in the fundus, ultrastructural analysis showed definite and highly significant lesions in the normally close associations between IC-IM and enteric nerve terminals. Breakdown of these structures, even without loss of IC-IM, could explain the reduced neural responses in the diabetic stomach. Taken together, these observations support the idea that loss of ICC, discontinuities in ICC networks, or breakdown in the synapse-like contacts between ICC and enteric neurons may play a major role in the pathogenesis of diabetic gastroparesis.

Because gastric complications of diabetes have not previously been documented in NOD mice, we characterized some of the key features of gastric dysfunction associated with diabetic gastropathy and gastroparesis. Gastroparesis, the irreversible end-stage form of diabetic gastropathy, is defined as slower-than-normal emptying of gastric contents (1–4). Currently, the most commonly used parameter to quantify gastric emptying is its half-time, and this factor is determined by scintigraphic analysis (36). This technique has not yet been applied to mice, and therefore we used a previously described technique for testing gastric emptying in this species (21,22). This technique is based on measuring the contents of the stomach remaining at intervals after a test meal and expressing this weight as a percentage of the total weight of the meal. To improve the accuracy of the measurements, we administered the preweighed food to fasted animals by

FIG. 3. Effects of diabetes on antral ICC networks: transmission electron micrographs of the circular muscle layers (A and B) and the myenteric regions (C and D) of the antrums of nondiabetic (A and C) and diabetic (B and D) NOD mice. ICC were identified by ultrastructural features (electron-dense nucleus with heterochromatin distributed toward the periphery of the nuclear envelope, electron-dense cytoplasm, numerous mitochondria, membrane caveolae, well-developed rough endoplasmic reticulum, Golgi complexes, and an incomplete basal lamina) (13,15,17,20,31,32). In nondiabetic muscles, IC-IM (A) were closely associated with nerve fibers (*) in nearly every section. In diabetic animals, enteric nerve fibers and bundles were not usually associated with IC-IM in either the circular or longitudinal muscle layers (B) (circular muscle layer shown). In the myenteric region, the ICC remaining in the diabetic muscles (D) were frequently separated from enteric neurons by wide extracellular spaces (ecm; G, enteroglial cell). Perinuclear cytoplasm was markedly reduced in IC-MY. Scale bars in control images apply to corresponding images from diabetic tissues.
gavage and calculated residual gastric contents after vari-
ous periods of time. We found that essentially all gastric con-
tents were emptied within 3 h in BALB/c and nondiabetic 
NOD mice. In diabetic mice, there was a significant reduction 
in the amount of food emptied during the 3-h test period, sug-
gesting impairment in gastric emptying in these animals.

Normal gastric emptying requires the proper function of the 
gastric electrical pacemaker system and the coordination 
between gastric peristaltic contractions and openings of the 
pyloric sphincter (1). Gastroparesis has been associated with 
electrical abnormalities (1,6–8), and deviations from normal 
slow-wave rhythm (dysrhythmias) have been reported to 
result in delayed gastric emptying (8). Abnormal slow-wave 
activity has also been reported in the antrums of rats with 
streptozotocin-induced diabetes. (23). In these studies, most 
strips of antral muscle from diabetic animals were electrically 
quiescent; however, a few strips retained slow waves of 
slightly reduced amplitude and frequency (but these changes 
were not significantly different from nondiabetic muscles) 
(23). Electrical rhythmicity in the stomachs of NOD diabetic 
mice was similarly affected. However, we also noted a gra-
dient in the lesion in which the corpus was relatively normal 
and the antrum was profoundly abnormal. Diabetes and the 
development of associated gastropathy are progressive dis-
orders, and therefore it may not be surprising that patches of 
antral muscle were still relatively normal, whereas other 
regions were significantly impaired.

Besides the generation of slow waves, pacemaker ICC (in 
this case, IC-MY) are responsible for another fundamental 
property of electrical rhythmicity, active propagation. Smooth 
muscle cells lack the ionic mechanisms necessary for slow-
wave regeneration (12,37). Thus, propagation of slow waves, 
a critical feature of normal gastric motility, requires intact 
IC-MY networks throughout the phasic regions of the stomach. 
This point is reinforced in the present study because pace-
maker activity was not uniformly lost in the stomach. Most 
of the corpus and even patches of the antrum retained slow-
wave activity. The important point is that while pacemaker 
activity was found in parts of the stomach, slow waves did not 
propagate throughout the entire stomach. Thus, even the 
patchy loss of IC-MY observed in NOD diabetic mice would 
lead to incomplete electrical pacing of the tunica muscularis 
and abnormal gastric motility. Loss of connectivity between 
portions of the IC-MY network could lead to ectopic and mul-
tiple pacemaker sites, which could appear in whole-organ or 
extracellular electrical recordings as various patterns of elec-
trical dysrhythmia. It seems rather likely that loss of regener-
ative capacity for slow waves in the antrum and defective 
propagation could contribute significantly to gastroparesis.

Despite the widespread occurrence of gastropathy 
among diabetic patients, not all patients with clinical symp-
toms have delayed gastric emptying (1–3,38), and there is a 
striking dissociation between dyspeptic symptoms and 
gastric stasis (2,38). It has been suggested that abnormali-
ties in fundic tone may contribute to dyspepsia-like symptoms experienced by diabetic patients (1,27). Indeed, in diabetic patients, fasting fundic tone is lower (24) and does not relax in response to distension (25), resulting in reduced retention of gastric contents in the proximal stomach (24,26). Impaired receptive relaxation could be due to reduced nonadrenergic noncholinergic inhibitory neural control of the proximal stomach, and reduced inhibitory neural responses have been reported in studies of streptozotocin-induced diabetic rats (23). Other studies have suggested that reductions in nitric oxide synthase expression and loss of nitrergic neural control could underlie these changes (9,10). Loss of IC-IM could also contribute to the pathogenesis of impaired control of fundic tone. The close association between IC-IM and enteric neurons and gap junction connections between smooth muscle cells and IC-IM provide the structural basis for the role of IC-IM in neurotransmission. Indeed, physiological studies have shown these cells to be critical for both inhibitory and excitatory neurotransmission in the stomach (13–15), and there is even the suggestion that ICC could serve as amplifiers in enteric inhibitory neurotransmission (16,39). The most convincing evidence for the importance of ICC in neurotransmission comes from studies of murine mutants that lack IC-IM in the fundus because of impaired Kit signaling. Loss of IC-IM led to a dramatic reduction in the amplitudes of nonadrenergic noncholinergic (nitric oxide-dependent) inhibitory neurotransmission (13,14) and cholinergic excitatory neurotransmission (15). It should be noted that the reductions in IJPs in muscles of W/W° mice were similar to the changes observed in the fundus of streptozotocin-diabetic rats (23). In the present studies, we have found a reduction in both EJPs and IJPs, which would occur if the lesions in diabetic mice extended beyond changes in nitrergic neurons and included defects in IC-IM. The reduction in neurotransmission in diabetic mice appeared to be due to more subtle changes in IC-IM than in W/W° mice. In the latter, all IC-IM were missing from the stomach, and it seemed obvious that loss of neurotransmission could be caused by the loss of these cells. In diabetic mice, IC-IM (as shown by Kit-LI) were apparently still in normal abundance, but closer ultrastructural investigation detected important changes. The normal close contacts between IC-IM and enteric nerve terminals were much less frequent, and large spaces of the extracellular matrix separated IC-IM from nerves. This breakdown in what appears to be the requisite neuromuscular junction in the stomach may explain the reduced neural inputs (both excitatory and inhibitory) in diabetic mice.

Delayed gastric emptying can also result from impaired pyloric relaxation or abnormal antroduodenal coordination (1). Indeed, nitrergic neurons, which can be affected by diabetes (9,10), play an important role in the inhibitory control of pyloric motility (14). In the pyloric sphincter, ICC serve as mediators of nitrergic inhibitory neurotransmission (14), raising the possibility that these cells could also be involved in this aspect of diabetic gastroparesis. Additional studies are needed to monitor ICC populations and the consequences to pyloric function of changes in these cells.

A surprising finding in this study was the presence of electrical rhythmicity in the (normally quiescent) fundus of some diabetic mice. Phasic electrical activity was accompanied by the abnormal presence of IC-MY networks in the fundus (11–13). Such a defect would lead to loss of normal electrical propagation, which is fundamental to gastric peristalsis. Breakdown in the close associations between IC-IM and nerve terminals might compromise neural responses in the diabetic stomach and lead to the loss of receptive relaxation and neural control of motil-
ity. We suggest that loss of ICC, remodeling of ICC networks, and breakdown in nerve/ICC associations may be responsible for some of the major symptoms of diabetic gastropathy and gastroparesis.

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