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Diabetic gastrointestinal motility disorders and the role of enteric nervous system: Current status and future directions

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Abstract

Gastrointestinal manifestations of diabetes are common and a source of significant discomfort and disability. Diabetes affects almost every part of gastrointestinal tract from the esophagus to the rectum and causes a variety of symptoms including heartburn, nausea, vomiting, abdominal pain, diarrhea and constipation. Understanding the underlying mechanisms of diabetic gastroenteropathy is important to guide development of therapies for this common problem. Over recent years, the data regarding the pathophysiology of diabetic gastroenteropathy is expanding. In addition to autonomic neuropathy causing gastrointestinal disturbances the role of enteric nervous system is becoming more evident. In this review, we summarize the reported alterations in enteric nervous system including enteric neurons, interstitial cells of Cajal and neurotransmission in diabetic animal models and patients. We also review the possible underlying mechanisms of these alterations, with focus on oxidative stress, growth factors and diabetes induced changes in gastrointestinal smooth muscle. Finally, we will discuss recent advances and potential areas for future research related to diabetes and the ENS such as gut microbiota, micro-RNAs and changes in the microvasculature and endothelial dysfunction.

Keywords

Diabetes; Enteric nervous system; Enteric neurons; Smooth muscle; Interstitial cells of Cajal; Oxidative stress

Introduction

The enteric nervous system (ENS) is an independent network of neurons and glial cells that is responsible for controlling the gastrointestinal (GI) tract's functions including motility, secretion and participation in immunoregulation[1–3]. ENS is structured as two major plexi, myenteric and submucosal, formed by small ganglia and neurons connected through bundles of nerves that run in the course of the entire GI tract. This system is connected to the central nervous system via sensory neurons that send afferent fibers conveying visceral sensations such as pain, stretch, fullness and nausea as well as efferent sympathetic and parasympathetic pathways that modulate motility, secretion and circulation[4]. In addition to the enteric neurons, interstitial cells of Cajal (ICC) are non-neuronal, non-glial cells that are

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present throughout the GI tract within multiple layers of the esophageal, gastric and intestinal wall including the myenteric plexus[5]. These cells function as the pacemaker, generating electrical activity resulting in the slow wave peristaltic movement of the intestine[6, 7]. Furthermore, they are involved in neurotransmission between enteric motor neurons, efferents from central nervous system and smooth muscle cells in the wall of GI tract[8, 9].

Diabetes mellitus (DM) is commonly associated with gastrointestinal symptoms such as nausea vomiting, diarrhea, abdominal pain and constipation[10, 11]. Diabetes affects almost all parts of the GI tract but the exact prevalence of diabetic gastroenteropathy is unclear[12, 13]. In the esophagus, DM is associated with decreased baseline tone of lower esophageal sphincter (LES), possibly leading to increased gastroesophageal reflux disorder (GERD)[14]. It has been reported that esophageal motility is delayed in patients with DM with impaired peristaltic movements in the esophagus[15]. The prevalence of silent esophageal dysmotility due to DM is reported to be higher than what is reported by the patients based on their symptoms[16, 17]. In the stomach, a wide variety of disturbances in the gastric motility as a result of DM has been reported. DM has been linked both to rapid gastric emptying, especially in the early stages of the disease[18], and more commonly with delayed gastric emptying and gastroparesis[17, 19]. Impaired relaxation of the gastric fundus has been reported to be accountable for early satiety and dyspeptic symptoms[20, 21]. Electrophysiological studies have shown dysrhythmias of slow wave contraction, prolonged pyloric contractions, and impaired coordination between antrum and duodenum[22–24]. The effect of diabetes on small intestine and colonic functions is not as well studied. Increased prevalence of both constipation and diarrhea is reported in patients with DM. While initial data suggested that the intestinal transit time is slowed in animal models of DM leading to bacterial overgrowth and subsequently diarrhea[25], further studies showed the presence of accelerated intestinal transit time in some models[26]. This accelerated intestinal transit was attributed to autonomic neuropathy and DM induced denervation of sympathetic nerve terminals. Colonic transit time is often increased and constipation is a common complaint in patients with DM[27]. The major changes in motility in various parts of the GI tract are shown in Figure 1.

Our understanding of the underlying mechanisms of DM induced changes in GI tract has changed over the recent years. It was a widely accepted view that autonomic neuropathy is the underlying mechanism of gastrointestinal manifestations of DM; however, emerging evidence has suggested that other pathophysiologic factors also play an important role. Disturbances in the enteric nervous system and ICC, independent of autonomic nervous system, and smooth muscle myopathy are among these emerging mechanisms. In this review, we highlight recent studies using animal models and human tissue to investigate the effect of diabetes on inhibitory and excitatory neurons, population of ICC, interactions between enteric neurons and ICC in neurotransmission, and smooth muscles. Additionally, mechanism of altered survival of enteric neurons and ICC in DM such as oxidative stress and growth factors are elaborated.

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Animal Models of Diabetic Neuropathy

Over the past few decades there have been several animal models developed to study diabetic neuropathy[28]. The most commonly used models of diabetic neuropathy include genetically modified[29, 30], Streptozotocin-induced[31] and high-fat diet induced mouse models[32]. One of the advantages of the classic Streptozotocin-induced diabetes model is that the animal develops a neuropathy similar to human diabetic neuropathy with features such as reduced size of nerve fibers, axon and myelin sheath and decreased nerve conduction velocities[31]. However, it has been suggested that toxic effects of Streptozotocin rather than diabetes is responsible for some degree of neuropathy observed in this model[33]. For studying human sensory neuropathy, one of the better models is the Streptozotocin-induced diabetic sensory neuropathy ddY mouse model, in which the mice have a lower sensory nerve conduction velocity, higher nociceptive threshold, hypoalgesia and unmyelinated fiber atrophy[34]. Spontaneously diabetic WBN/Kob rat model is another animal model used to study diabetic neuropathy with the advantage that it resembles the human pathogenesis of diabetic neuropathy with structural de-and remyelination in the sciatic and tibial nerves at 12 months without confounding effects of streptozotocin. At 20 months of age these mice have axonal degeneration, dystrophy and reduced myelinated fibers[35]. Similarly, the Nonobese diabetic (*NOD*) mouse model[29, 30] and the Leptin-deficient *ob/ob* mouse model[36] are consistent with the human pathogenesis of human peripheral diabetic neuropathy. Finally the high-fat diet-fed mouse model does demonstrate evidence of motor and sensory nerve conduction deficits and can be used as a model of obesity-related neuropathy[32]. In summary, some of the models most applicable to human diabetic neuropathy include the Streptozotocin-induced diabetic mouse models as well as the genetically modified NOD and *ob/ob* mouse models. These models have frequently been used to study diabetes induced enteric neuropathy.

Diabetes and autonomic neuropathy

The gastrointestinal tract is heavily connected to autonomic nervous system. Almost all parts of GI tract receive efferent connections from sympathetic and parasympathetic fibers and send afferents to the parasympathetic system. In the light of this interconnection and well-known autonomic neuropathy caused by diabetes, autonomic neuropathy was considered the origin of GI manifestations of DM. In diabetic patients, vagal nerve fibers show evidence of segmental demyelination and axonal degeneration both within myenteric and submucosal plexi and outside of the GI tract[37, 38]. Structural changes in axons of vagal fibers are seen in spontaneous diabetic rats[39]. In both patients and animal models of diabetes, the number of cells in motor vagal ganglions and sensory sympathetic ganglions is reduced[40–42]. However, the clinical correlation between GI symptoms and other evidence of autonomic neuropathy such as increased variability of R-R interval on electrocardiogram is controversial[15, 43]. Additionally, some studies have reported that although the number of neurons in the sympathetic and parasympathetic ganglions is reduced and there are structural changes in the axons, the overall density and morphology of vagal efferent fibers is not changed in animal models of diabetes[44]. It has been shown that vagal afferent fibers are closely related to ICC and express nNOS. A decrease in nNOS expression in the afferent vagal nerve has been reported in rat model of DM[45]. These findings suggest that most of the changes in

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diabetes in the autonomic nervous system might be related to the afferent arm of the gut-autonomic nervous system connection.

Diabetes and enteric neuropathy

The effect of DM on the population of enteric neurons is mostly studied in the rodent model of streptozotocin (STZ)-induced type I DM. Several of these studies have shown a reduction in number of enteric neurons in most parts of the GI tract including stomach[46], ileum[47, 48], cecum[49], and colon[48, 50, 51]. Degenerative structural changes such as axonal swelling have also been observed as early as 2 weeks after the onset of diabetes[52]. Similar reduction in the number of enteric neurons has been shown in spontaneously diabetic rats[53, 54] and non-obese diabetic (NOD) mice[55, 56]. Interestingly, DM might preferentially affect inhibitory neurons more than excitatory neurons. The population of nitrergic neurons is affected early after the onset of DM in animal models and expression of nNOS is reduced in diabetic animals while the population of cholinergic enteric neurons remains unaffected until later in the course of DM[57]. In a study of colonic tissue obtained from human subjects with DM, a decrease in the number of nitrergic neurons as well as neurons containing neuropeptide Y, another inhibitory neurotransmitter, but not in the number of cholinergic neurons has been reported[58]. Another study examined the population of nitrergic neurons in the appendix of 6 patients with type 1 DM and reported a significant decrease in the number of nNOS containing neurons within the myenteric plexus as well as muscular layers[59].

Mechanism of Diabetic enteric neuropathy—The mechanism of degeneration of enteric neurons in DM is not clear. Some studies using STZ model and cell cultures have suggested increase in apoptosis due to hyperglycemia similar to the effect of hyperglycemia on other neuronal cell types[41, 60, 61]. Enteric neurons are shown to be glucose responsive and it is possible that hyperglycemia will activate apoptotic pathways by causing hypercalcemia due to increased activation of ATP-sensitive K+-channels[62, 63].

Another mechanism suggested to play a role in decreased population of enteric neurons is decreased neuronal growth factors due to DM. Enteric neurons originate from vagal and sacral neural crest cells and they express Ret tyrosine kinase receptor which is essential for their differentiation, migration and survival. Glial cell line-derived neurotrophic factor (GDNF) is a growth factor that activates this receptor and through PI3K and MAPK pathways[64–66]. Diabetes is known to reduce the transport and function of nerve growth factors and it is possible that decreased survival of enteric neurons is accentuated by lack of enough growth factors. Furthermore, Insulin like growth factor-1 (IGF-1) and neurotrophin-3 (NT-3) have been reported to successfully reverse degenerative changes of nerves in animal models of diabetic neuropathy[67–69]. We have reported that hyperglycemia causes reduced activation of PI3K in cultured embryonic enteric neurons and in STZ – induced rat model of DM, leading to increased apoptosis of enteric neurons. In this experiment, GDNF was protective against adverse effects of hyperglycemia on enteric neurons and its addition to the culture led to significant reduction in apoptosis[60]. The number of enteric glial cells is also reduced in animal model of diabetes. It has been reported that the number of S-100 expressing enteric glial cells in the stomach of STZ-induced rat model of diabetes is reduced in correlation with development of delayed gastric emptying.

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Diabetic patients have high circulating levels of free fatty acids[70]. In mice fed with high fat diet and subsequently developed DM, the number of enteric glial cells was reduced in small intestine prior to loss of enteric neurons[71]. We and others have also shown that a diet high in fat can lead to intestinal enteric neuronal loss[72, 73]. Palmitic acid seems to play an important role in mediating this damage. Another growth factor signaling pathway that has reported to be altered in animal model of diabetes is transforming growth factor (TGF) beta. One recent study investigated the role of bone morphogenetic proteins (BMPs), a molecule involved in TGF beta signaling pathway and gut morphogenesis, in loss of enteric neurons in animal model of diabetes. In an experimental rat model of diabetes, abnormal BMP2 signaling was found in the myenteric plexus which was restored with insulin therapy. This suggests that the BMP2/SMAD signaling pathway is important in diabetic enteric neuropathy[74].

Oxidative stress which results from excess of reactive oxygen species (ROS) has also been suggested as a potential mechanism of enteric neuron degeneration as a result of DM. In animal models of DM, increased oxidative stress has been shown to be associated with gastroparesis and treatment with antioxidants can prevent the development of gastroparesis[75]. In the high fat diet fed mouse model excessive palmitoylcarnitine formation leading to energy depletion and oxidative stress are felt to be one of the mechanisms underlying neuronal loss[72, 73]. Quercitin is a powerful antioxidant that has been shown to prevent the reduction in enteric neuronal numbers in the cecum of diabetic rats[76]. Examination of enteric ganglia obtained from colon biopsy specimen of diabetic patients through laser capture microdissection showed decreased levels of antioxidant GSH and increased expression of superoxide dismutase (SOD) mRNA which indicate increased oxidative stress. This increase in oxidative stress seems to preferentially affect the inhibitory nitrergic neurons, evident as decreased contractility in response to nitric oxide inhibitor. Addition of antioxidant lipoic acid to the cultured enteric neurons exposed to hyperglycemia reverses the reduction in PI3K activation, providing further evidence for increased oxidative stress due to hyperglycemia[58]. Furthermore, *in vitro* evidence suggests that GSH from enteric glia might have a protective effect on enteric neuronal survival[77]. In mouse models of diabetes, increased expression of antioxidants such as HO-1 can protect mice from developing gastroparesis[75]. In STZ-rat model of DM, nitrergic enteric neurons that also express HO-2 are more resistant to the effects of DM and are less likely to undergo apoptosis[78]. However, trials of antioxidants in diabetic patients have not been very successful in preventing or improving gastroparesis[79], suggesting the need for more studies to develop the optimal therapeutic approach.

There are other suggested but not well-studied mechanisms for enteric neuropathy in diabetes. We will mention two of these mechanisms briefly here: accumulation of N-acetylaspartic acid and S-nitrosylation reactions. Normal hydrolysis of N-acetylaspartic acid (NAA) is important to maintain enteric neurons. Aspartoacylase (ASPA) is the enzyme responsible for hydrolysis of NAA and it has been shown that in an obesity-induced mice model of diabetes, the activity of this enzyme reduced, leading to accumulation of NAA and potentially contributing to loss of enteric neurons[3, 80]. One of the signaling pathways of NO is through S-nitrosothiol (SNO). In the central nervous system, aberrant S-nitrosylation of some molecular targets is implicated in CNS pathology but the role of abnormal SNO

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signaling in enteric nervous system is not clear. Recently, it has been shown that S-nitrosylation reactions may be involved in toxin-induced neurogenic inflammation in the colon[81]. The role of these factors in diabetes induced GI dysmotility remains to be elucidated through further research.

DM and Interstitial Cells of Cajal

ICC are non-neuronal cells that are present in multiple layers of GI tract's wall and are named based on their location. For example, a group of ICC located in the myenteric plexus is named ICC-MY and a group of them located in muscular layer is called ICC-IM[82]. Recent evidence has suggested that these cells are different in ultra-structural morphology and function in addition to location[5, 83]. Development of ICC, like enteric neurons, is dependent on a tyrosine Kinase receptor, Kit[84]. Primary evidence for role of ICCs in control of GI motility came from animal models in which Kit receptors were blocked. These animals developed severe GI motility dysfunction with absence of slow phase peristaltic movements[85, 86]. Since then, ICCs became an area of interest in studies investigating abnormalities in GI motility, including those caused by DM and several other functions have been attributed them such as mediating neurotransmission between autonomic nervous system and enteric neurons or between enteric neurons and smooth muscles[9, 87, 88].

Studies in animal model of DM and gastroparesis have shown that populations of ICC-MY and ICC-IM in gastric antrum and ICC-IM in gastric fundus are reduced in STZ model of DM as well as NOD model[89, 90]. The reduced population of ICC-IM in fundus is proposed to be associated with impaired relaxation of gastric fundus in diabetic animal, while reduced population of ICC-MY is associated with impaired slow wave peristaltic movements and delayed gastric emptying[91]. A case report of a patient with insulin-dependent DM with gastroparesis, demonstrated decreased population of ICC-IM in human, similar to animal models[92]. Foster et al reported the results of gastric biopsy from 14 patients with gastroparesis including 9 patients with DM and showed reduction in the number of ICC[93]. In 25 patients with DM and gastric cancer who underwent gastrectomy, study of the gastric tissue showed reduced number of ICC in the inner circular muscle layer but not the myenteric plexus[94]. Diminished population of ICCs has been reported in other parts of GI tract in animal model of diabetes including colon and small intestine. A case-series of 7 patients with DM showed reduced number of ICC in colonic tissue[95]. Yamamoto et al, using a db/db model of type 2 DM, showed reduced number of ICC in small intestine and colon in addition to stomach[96].

In addition to being an independent pulse maker for slow wave movements of GI tract, ICC appear to be an important part of neurotransmission between motor neurons, efferent input from autonomic nervous system and muscle fibers. It has been proposed that both excitatory and inhibitory neurotransmission between enteric neurons and smooth muscles are dependent on presence of ICC-IM. Morphologically, there is close contact with synaptic specification between ICC and enteric neurons[9, 88, 97]. It has been shown that acetylcholine released from enteric motor neurons attaches to the Ach receptors of ICC-IM which in turn depolarize the smooth muscles through gap junctions[8]. The modulatory role of ICC-IM has also been shown in excitatory neurotransmission through substance P and neurokinin[98]. As

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for inhibitory neurotransmission, ICC-IM in distal esophagus and antrum of mice are surrounded by NO-containing nerves and mice model lacking ICC-IM doesn't show any evidence of nitrergic transmission[87]. Therefore, it is possible that reduced population of ICC-IM impairs neurotransmission, adding to the disturbed motility caused by impaired pacemaker activity from ICC-MY. However, there are remaining questions about the degree that neurotransmission happening through ICC is important in controlling GI motility. One important reservation is that the number of ICC is considerably lower that smooth muscle cells, suggesting that neurotransmission cannot rely solely on ICC[5].

Mechanism of DM induced changes in ICC—Similar to the enteric neurons, the mechanism by which DM affects ICCs remains to be fully elucidated. One interesting finding reported in the literature is that hyperglycemia alone is not enough to affect ICC and in fact lack of Insulin and Insulin-like growth factor-1 (IGF-1) plays the major role in reduced survival of ICC[99]. However, ICC lack receptors for insulin of IGF-1 and instead have receptors for stem cell factor (SCF). On the other hand, smooth muscle cells and enteric neurons have receptors for insulin and IGF-1. It has been suggested that ICC require SCF secreted by smooth muscle cells and enteric neurons in response to Insulin. Lack of insulin, reduced population of enteric neuron as the result of DM and myopathic changes in smooth muscle cells can all lead to decreased amount of CSF and consequently diminished survival of ICC[100]. While this finding might provide an explanation for long-term effects of diabetes on GI motility through its effect on population of ICC, it does not explain the reported effect of acute changes in serum glucose level on gastric motility. Hyperglycemia and hypoglycemia have both shown to change the rate of gastric emptying acutely[101]. This might be due to dysrhythmia in the pacemaker activity of ICCs or change in the contractibility of smooth muscle fibers.

DM and the balance of excitatory and inhibitory neurotransmitters

The main excitatory neurotransmitter of enteric nervous system is acetylcholine followed by neurokinin and substance P that are released by enteric motor neurons and have receptors on ICC-IM, mediating the contraction of smooth muscles[102]. Inhibitory neurotransmission in enteric nervous system happens through non-adrenergic non-cholinergic (NANC) pathway. Nitric oxide (NO) is the main inhibitory neurotransmitter, with neuronal nitric oxide (nNOS) being the rate controlling enzyme in its production within ENS[103, 104]. Studies of animal models with DM have demonstrated that the sources of nitrergic nerve terminals are both from intrinsic motor neurons and vagal and parasympathetic afferents. The distribution of nitrergic neurons is not equal throughout the GI tract, with them being more prevalent in the stomach and proximal parts of the intestine[105, 106].

In animal models of DM, there is a gender specific reduction in the expression of nNOS and the production of NO within ENS[107, 108]. One study in STZ-induced diabetes in rats showed that there is reduction of NO in nerve terminals within the muscular layer of GI wall without decrease of nNOS positive neurons in parasympathetic ganglions. The authors suggested that there might be a diabetes-induced impairment of transport of nNOS from cell bodies to the nerve terminals[56]. The attenuation in expression of nNOS mRNA and production of NO in diabetic rats is associated with impaired relaxation of gastric muscles and subsequently

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decreased gastric capacity and probably delayed gastric emptying. In STZ-rat model of diabetes, depletion of neurotransmitter containing vesicles occurs before destruction of the enteric nerve terminals in the gastric tissue and application of electrical stimulation early in the course of the disease can partially reverse this process[109]. Animal models have also shown that reduction of nNOS expression in the stomach is a gradual process that happens earlier than loss of acetylcholine positive nerve endings and is reversible with early insulin treatment. A two-phase model of NO depletion has suggested in which during the first phase, expression of nNOS is reduced in a reversible fashion that if not treated, will eventually lead to irreversible loss of nitrergic neurons[110, 111]. Furthermore, DM can disturb the balanced expression of other neuropeptides such as vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP) and substance P. In the ileum of diabetic rat, electrical stimulation failed to increase the release of VIP and CGRP, whereas endogenous release of acetylcholine, serotonin, and substance P was increased similar to normal animal[112]. The imbalance between expression of enteric neuropeptides is reported to be reversible with insulin treatment[113].

Few studies in human patients with DM have confirmed the findings from animal models. Studying the gastric tissue obtained from patients with gastric cancer and DM revealed that the expression of nNOS is markedly decreased in the antrum, especially in the areas that have reduced density of ICC[94]. In another study, we examined the colonic tissue obtained during colonoscopy from patients with and without diabetes and demonstrated that the population of nNOS containing enteric neurons is reduced. In addition to NO, other inhibitory neurotransmitters such as neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP) are also reported to be reduced in DM[58].

Mechanism of DM induced changes in neurotransmitters—The mechanism of reduced expression of nNOS is not fully understood. Earlier data has shown that the loss of nitrergic nerves is independent of sympathetic and parasympathetic input. Only afferent vagal nerves appear to contain NO that located in close proximity with ICC-IM. It has been suggested that these afferent vagal nerve endings could be mechanoreceptors and their survival is mutually dependent on ICC-IM. Loss of ICC as a result of diabetes can cause the loss of afferent vagal nitrergic nerve endings and vice versa[45]. Another possibility is the loss of inhibitory enteric neurons that express high levels of nNOS. Evidence has suggested that inhibitory enteric neurons are more susceptible to the effects of diabetes and their population is reduced earlier in the course of the disease[58]. Others have proposed that non-enzymatic glycosylation of proteins that leads to production of advanced glycation end-products (AGEs) might play a role in reduced expression of nNOS, since inhibiting these products can prevent the depletion of NO from nerve terminals or even restore the depleted amount[61, 114].

DM and GI smooth muscle

Contractility of smooth muscle cells is altered in animal models of DM. Myocytes isolated from GI tracts of STZ-induced rat show increased expression Na, K-ATPase, leading to increased intracellular calcium level[115]. In the same animal model, intracellular calcium binding proteins such as calmodulin and protein kinase C in intestinal smooth muscle cells

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are reduced. Similar changes are found in spontaneous diabetic rats, suggesting that diabetes alters smooth muscle contractility through changing the intracellular signaling pathways of intestinal myocytes[116]. Another study, using both STZ-induced DM and spontaneous DM models, suggested that impairment in the function of GTP-binding proteins might be important in altered contractility of gastric smooth muscles, providing further evidence for the importance of DM induced changes in the intracellular signaling pathways[117]. Myopathy and atrophy of gastric smooth muscle has also been reported[100, 118]

Another mechanism that is proposed to be involved in pathogenesis of DM-induced dysmotility is the change in phosphorylation of myosin light chain (MLC). Phosphorylation of MLC is an important regulatory mechanism in contraction of intestinal smooth muscles. Two important enzymes are involved in the process of phosphorylation (myosin light chain kinase (MLCK)) and dephosphorylation (myosin light chain phosphatase (MLCP)) of MLC. Targeted deletion of MLCK leads to significant disturbance in GI motility[119]. In a study of STZ-DM rats, the expression of MLCK was significantly reduced in the smooth muscles of pylorus and ileum. This effect was reversible with insulin treatment[120].

Other Factors and New Directions

Autoimmunity

Lymphocytic infiltrates have been reported in MP of some animal models of DM[52], but no clear correlation between presence of these infiltrates and DM-induced GI disturbance has been proved yet. In patients with DM, results have been contradictory. While inflammatory infiltrates have been reported in esophagus[38] and autonomic ganglia[121] of patients with diabetes, gastric specimens from diabetic patients with gastroparesis have not revealed any inflammation[118]. Efforts have been made to find functional antibodies that can act against enteric neurons or other parts of ENS in patients with diabetes and have been largely unsuccessful. There is a report of antibody against L-type calcium channels can disturb migrating myoelectric complex in mice[122], but further studies are required to verify the role of autoimmunity in pathogenesis of enteric neuropathy in diabetic patients.

Endothelial Dysfunction

Enteric neurons within MP receive blood supply from adjacent capillaries. A recent study has shown that endothelial cells in the capillaries close the enteric neurons are damaged in diabetic animals, leading to change in the permeability of these capillaries as well as altered expression of adhesion molecules. The diabetes-induced change in the capillaries of wall of the gut is not uniform within various segment of GI tract and has a distribution that resembles the distribution of changes in enteric neurons and nitrergic transmission[123]. This evidence might suggest a role for endothelial dysfunction in pathogenesis of changes in ENS as a result of diabetes.

Microbiota

The effect of gut's microbiota on GI motility is receiving more recognition due to recent research. It has been shown that elimination of microbiota causes dilatation of cecum as a result of abnormal motility that returns to normal when colonization of bacteria is

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allowed[124]. This change in GI motility has been attributed to change in neurotransmitters. It is well known that the composition of microbiota changes significantly in patients with DM and efforts have been made to explain the role of this change in pathogenesis of DM[125]. Some of the disturbances in GI motility such as diarrhea has been attributed to change in microbiota[25], but the exact role of altered microbiota in diabetes induced GI dysmotility is not studies. It is possible that a mutual relation between GI motility and change in gut's microbiata exists[126]. Decreased motility of GI tract can lead to change in the microbial flora of the gut while this change can alter neurotransmission within the gastric wall or even change the gastric motility through afferent signals to central nervous system[127–129].

Micro-RNAs

Micro-RNAs are small non-coding RNAs that post-transcriptionally regulate protein synthesis[130]. The role of miRNAs in regulating GI motility and survival of enteric neurons is becoming more evident from the recent evidence. For example, we have recently shown that loss of enteric neurons caused by high fat diet is mediated through Mir-357[72]. Role of miRNAs in organ damage caused by diabetes as well as pathogenesis of diabetes is being actively explored, but there is no data regarding role of miRNAs in diabetic induced changes of ENS. miRNAs are also involved in proliferation and development of gastrointestinal smooth muscles[131, 132]. Future research is necessary to identify miRNAs that are involved in toxic effects of hyperglycemia or lack of insulin on enteric neurons and smooth muscles since these can be potential therapeutic targets.

Autophagy

Autophagy is referred to the process of collecting damaged proteins and organelles in to autophagosomes. The primary purpose of this process is to protect cells from damage caused by malfunctioning proteins and organelles, but it also can lead to cell death in certain circumstances[133]. Impairment of autophagy along with increased oxidative stress is suggested to be involved in pathogenesis of DM as well as end-organ damage caused by DM[134]. An association between impaired autophagy and increased apoptosis in dorsal root ganglia has been reported in STZ-rat model of diabetes[135]. The role of impaired autophagy in the pathogenesis of diabetic gastroenteropathy has not been directly studied. However, we have reported that impaired autophagy might be important in damage to the nitrergic neurons caused by high-fat diet[72]. It is possible that with increased oxidative stress and damage to the proteins and mitochondria, an impaired autophagy will exacerbate the problem and will lead to accelerated death of enteric neurons. Future research is required to test this hypothesis.

Challenges and Future perspectives

One of the key challenges in treatment of diabetic induced enteric neuropathy is identifying new modifiable risk factors. There are risk factors that have been known for a while and strategies have been developed to modify them. These types of risk factors might include but are not limited to hypertriglyceridemia, obesity, dyslipidemia, and consumption of high-fat diet[136]. However, addressing these factors alone has not been sufficient to prevent enteric neuropathy caused by diabetes, suggesting that other factors are playing parts that are

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largely neglected in treatment protocols. For example, a recent study showed a beneficial effect of using human TNF-α receptor antibody in preventing diabetic neuropathy using a rodent model[137]. This study suggests that inflammation can play a key role in the pathogenesis of diabetic neuropathy. Oxidative stress is another important factor that is increased in diabetes and can lead to DNA damage, ER stress, mitochondrial complex dysfunction and neuronal apoptosis. The cells involved can include neurons, glia, vascular endothelial cells and can lead to the activation of macrophages leading to neuronal dysfunction and neuropathy[138]. Given the chronic progressive nature of the diabetic neuropathy, new treatment strategies may need to target multiple pathways contributing to the disease, including a combination of antioxidants, nerve growth factors and improvement of hyperglycemia and hyperlipidemia. Recently targeting ER stress was demonstrated to be beneficial in the treatment of diabetic neuropathy[139]. Novel research strategies need to focus on identifying potential inflammatory mediators of neuropathy as well as studying the role of non-coding RNAs (miRNAs and lncRNAs) in the pathogenesis of diabetic enteric neuropathy and their therapeutic potential[72]. Finally given the recent findings of the role of gut microbiota in contributing to the development of the metabolic syndrome, more research will need to be done in looking for potential changes in gut microbiota leading to progression of diabetic enteric neuropathy[126].

Conclusion

Recent developments in our understanding of how diabetes affects the enteric nervous system has caused a paradigm shift in how we think about GI manifestations of DM. Autonomic neuropathy is no longer considered the sole cause of GI dysmotility in DM and importance of enteric neurons, ICC, smooth muscle and enteric microbiota is becoming more evident. However, translating these findings into clinical treatment has been challenging. For example, while oxidative stress has been shown to play an important role in DM-induced apoptosis of enteric neurons and ICC[140], use of antioxidants with oral supplementation has not proved efficacious[79]. This calls for more research to find more creative approaches in delivering antioxidants to the enteric nervous system. Some steps have already been taken by using gene therapy in animal models to increase expression of antioxidants such as HO-1[75].

Another interesting recent development is a new line of research investigating the bidirectional interaction of microbiata and enteric motility[126]. Diabetes is known to change enteric microbiata and researchers were interested in this change from the perspective of DM pathophysiology. However, it has shown that some species of gut microbiota can be used to decrease oxidative stress. Additionally, metabolites produced by some of the species can stimulate or inhibit enteric nervous system and modulate GI motility[126]. Methods of altering microbi0ta in a favorable way to prevent and treat GI complication of DM remains to be fully developed with further research.

Another important question is how well the findings in animal models translate into human patients? So far, only few studies have used human tissues from diabetic patients to test the hypothesis driven from animal models[58, 92–95]. Although the findings of these studies have been mostly confirmatory of animal findings that diabetes reduces the number of enteric

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neurons and ICCs, there is no longitudinal data available to elucidate the chain of events that lead to GI complications of DM. Furthermore, there is no data to help predict which patient will develop GI manifestations and what the correlation between glucose control and GI dysmotility is.

In summary, DM significantly alters the microenvironment of enteric neurons and ICC, leading to decreased survival of the cells. Change of the microenvironment is multifactorial and includes increased oxidative stress, alteration of enteric microbiata, reduction of growth factors, change in intracellular signaling pathways and post-transcription regulatory factors such as miRNAs, and endothelia dysfunction (Figure 2). The interaction between these factors is complex, but each of them offers the potential for new therapeutic approaches.

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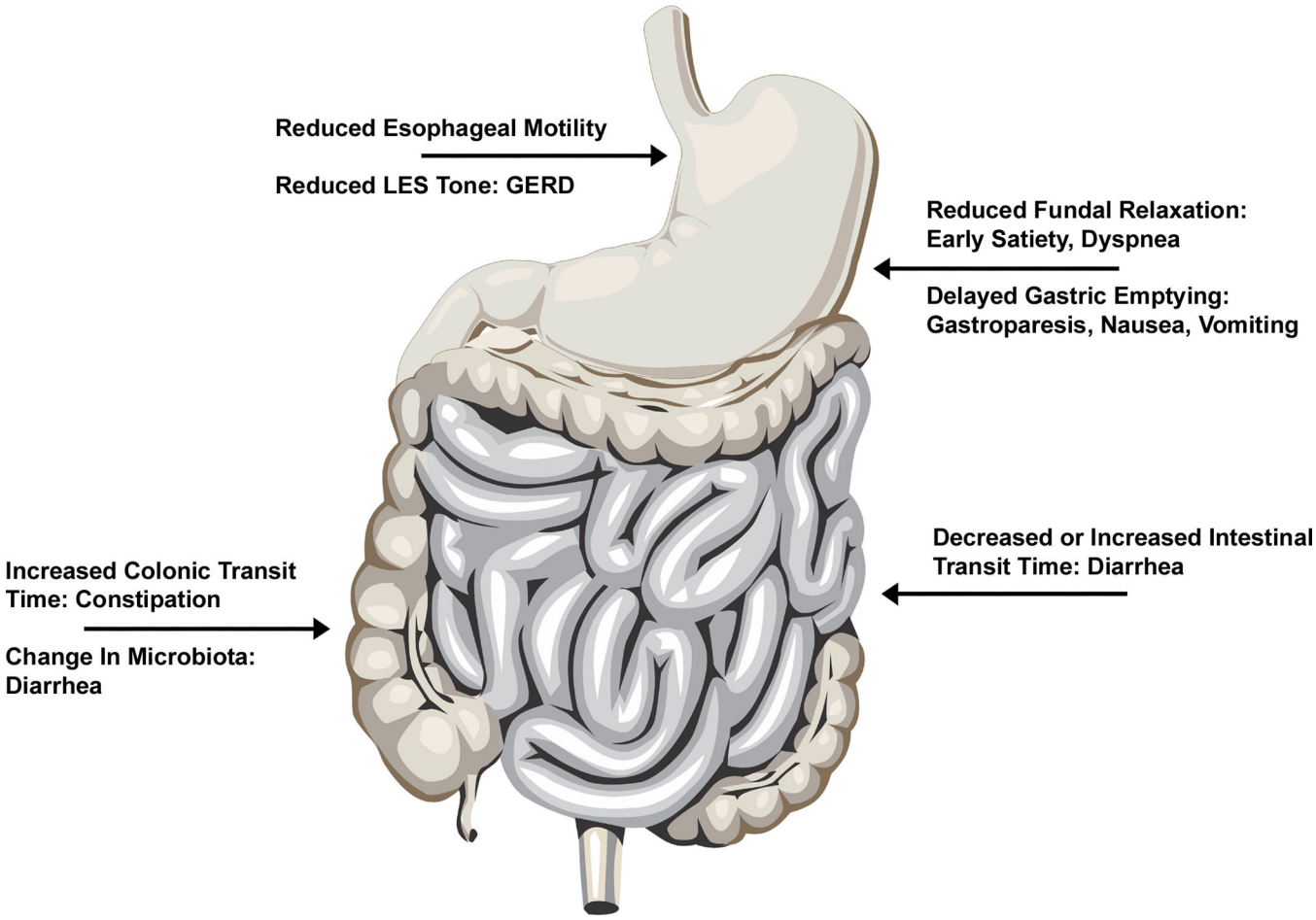

Figure 1. Effects of diabetes on motility in various parts of gastrointestinal tract

Diabetes affects almost all parts of GI tract. It can reduced the motility of esophagus and reduce the basal tone of LES. Gastroparesis and impaired gastric fundal relaxation are well known effects of diabetes on stomach. Both diarrhea and constipation are reported as GI manifestations of diabetes. GERD: gastroesophageal reflux disorder. LES: lower esophageal sphincter.

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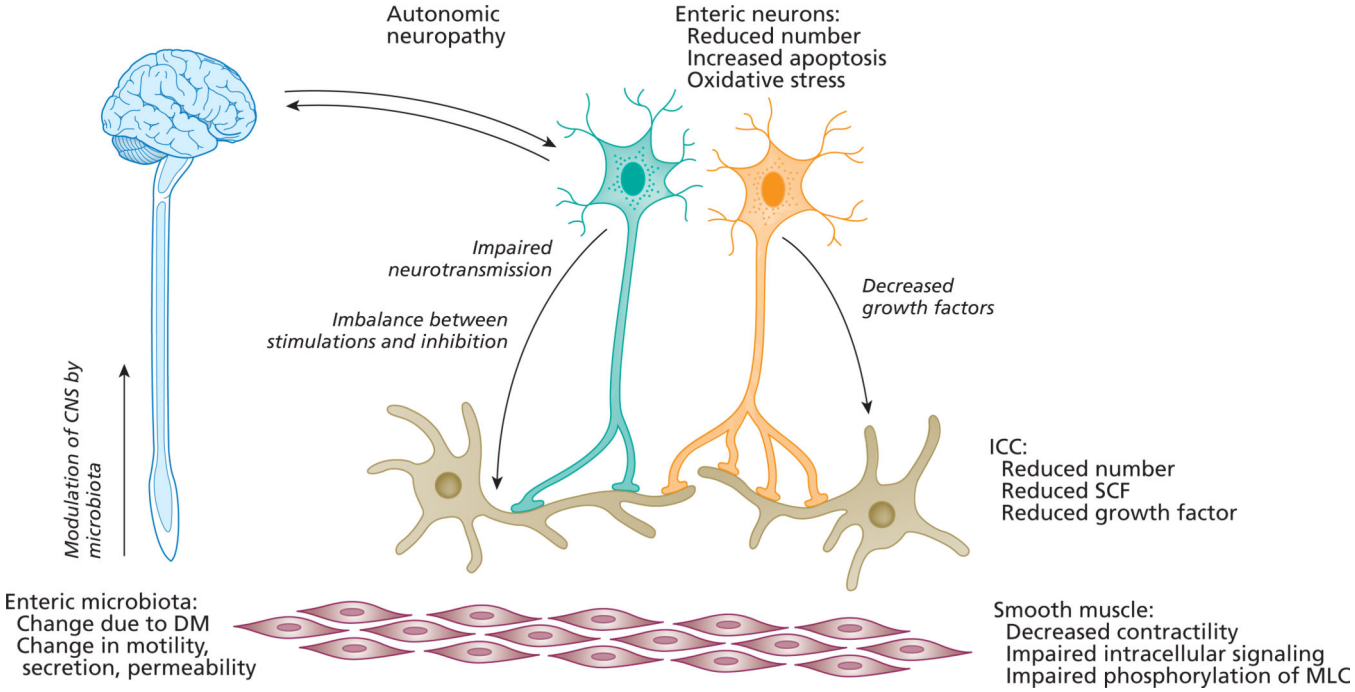

Figure 2. Potential mechanisms involving the ENS in impaired gastrointestinal motility in DM

Mechanism of diabetes induced gastrointestinal enteric neuropathy is complex and multifactorial. Autonomic neuropathy can affect both afferent and efferent connections between enteric nervous system and central nervous system. Reduced number of enteric neurons is caused by increased oxidative stress and increased apoptosis. The number of interstitial cells of Cajal in is also reduced, possibly due to lack of growth factors. There myopathic changes in smooth muscle and decreased contractility due to impaired signaling pathways. Diabetes induced changes in gut microbiota can modulate the CNS-gut interaction. ICC: interstitial cells of Cajal, SCF: stem cell factor, MLC: myosine light chain, DM: diabetes mellitus, CNS: central nervous system.