Musings on the Wanderer: What's New in Our Understanding of Vago-Vagal Reflexes? V. Remodeling of vagus and enteric neural circuitry after vagal injury

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Li, Ying, and Chung Owyang. Musings on the Wanderer: What's New in Our Understanding of Vago-Vagal Reflexes? V. Remodeling of the vagus and enteric neural circuitry after vagal injury. *Am J Physiol Gastrointest Liver Physiol* 285: G461–G469, 2003; 10.1152/ajpgi.00119.2003.—The vago-vagal reflexes mediate a wide range of digestive functions such as motility, secretion, and feeding behavior. Previous articles in this series have discussed the organization and functions of this important neural pathway. The focus of this review will be on some of the events responsible for the adaptive changes of the vagus and the enteric neutral circuitry that occur after vagal injury. The extraordinary plasticity of the neural systems to regain functions when challenged with neural injury will be discussed. In general, neuropeptides and transmitter-related enzymes in the vagal sensory neurons are downregulated after vagal injury to protect against further injury. Conversely, molecules previously absent or present at low levels begin to appear or are upregulated and are available to participate in the survival-regeneration process. Neurotrophins and other related proteins made at the site of the lesion and then retrogradely transported to the soma may play an important role in the regulation of neuropeptide phenotype expression and axonal growth. Vagal injury also triggers adaptive changes within the enteric nervous system to minimize the loss of gastrointestinal functions resulting from the interruption of the vago-vagal pathways. These may include rearrangement of the enteric neural circuitry, changes in the electrophysiological properties of sensory receptors in the intramural neural networks, an increase in receptor numbers, and changes in the affinity states of receptors on enteric neurons.

nodose ganglion; neurochemical transmission; enteric nervous system; gastrointestinal motility; pancreatic efferent signaling

THE VAGO-VAGAL REFLEXES SUBSERVE a wide range of gastrointestinal functions. It is well known that gastric relaxation occurs in response to esophageal, gastric, or duodenal distension (56, 16). Chemical stimulation of the duodenum with acid (35) or hyperosmolar solution (18) also inhibits gastric motility, which in turn contributes to delayed gastric emptying in the postprandial period. This enterogastric reflex activity coordinates the movement of chyme and maximizes the digestion and absorption of nutrients. The vago-vagal reflex pathways also participate in the control of food intake (51, 53) and in the control of gastric and pancreatic secretion (2, 32, 34). Hence, disruption of the vago-vagal pathways may result in marked disturbances of gastrointestinal functions. After chronic vagotomy, however, the digestive functions of the gastrointestinal tract usually are well preserved. This is due to the fact that the vagal pathways have an extraordinary plasticity to regain functions when challenged with injury. Vagotomy also may trigger adaptive changes within the enteric nervous system to minimize the loss of gastrointestinal functions resulting from the interruption of the vago-vagal pathways.

In this review, we will discuss the neural plasticity and regeneration after vagotomy or neuropathy. The role of neurotrophins in the survival and growth of the injured vagus nerve will be reviewed. Finally, we will discuss the adaptive changes that occur in the enteric nervous system to preserve digestive functions after chronic vagotomy.

NEURAL PLASTICITY AND REGENERATION AFTER VAGOTOMY

Vagal afferents vs. efferents. The vago-vagal neural circuit may be interrupted as a result of vagotomy or vagal neuropathies. Until the recent advent of powerful antiulcer drugs, truncal vagotomy as a means to reduce acid secretion was performed relatively frequently for peptic ulcer disease. Despite this, the digestive functions of the gastrointestinal tract usually were well maintained in most patients. This suggests that the vagal pathways have an extraordinary plasticity to regain functions when challenged with neural injury or the enteric nervous system is equipped to develop compensatory changes to preserve digestive functions.

Currently, little is known about the regenerative capacity of the vagus nerve. Most evidence for vagal reinnervation of the gastrointestinal tract after transection comes from function tests. For example, there are reports on recovery of vagally stimulated insulin secretion (47), gastric secretion (10, 41), pancreatic exocrine function (44), and gastric motility (7, 24, 41, 45). These observations are somewhat indirect because recovery may be mediated by compensatory mecha-

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nisms such as reorganization of the intrinsic enteric nervous system or hormonal responses.

Using anterograde tracing techniques, Phillips et al. (46) examined the terminal fields formed by regenerating axons and endings. These investigators reported marked differences in the regenerative capacities of the afferent and efferent arms of the vagus under the same surgical and maintenance conditions. It was demonstrated that, in the rat, vagal afferents regenerated by 18 wk after subdiaphragmatic transection to reinnervate the gut and to differentiate into the two types of terminals normally found in the smooth muscle wall of the gastrointestinal tract. Regeneration, however, is neither complete nor entirely accurate by 45 wk. Abnormal patterns of sensory organization occurred throughout the reinnervated field, with small bundles of axons forming complex tangles and some individual axons terminating in ectopic locations. The presence of growth cone profiles suggested that vagal reorganization was ongoing even 45 wk after vagotomy. In contrast, the vagal efferent fibers failed to reinnervate the gastrointestinal tract. Thus it appears that the vagal afferents have much greater regenerating capability compared with the efferent fibers.

Plasticity of the nodose ganglia neurons after axotomy. It is well established that primary sensory neurons demonstrate an extraordinary plasticity in their messenger molecule expression when challenged with nerve lesions (12, 15, 17, 48, 63, 64). These changes are usually referred to as the cell body reaction. In general, neuropeptides and transmitter-related enzymes in the sensory neurons are downregulated in response to nerve injury. Conversely, molecules previously not present, or present only at low levels, start to appear or become upregulated, and they may play a role in the survival-regeneration process (6, 37, 42).

With the use of immunohistochemistry and in situ hybridization, it was demonstrated that neuropeptides such as galanin, neuropeptide Y (NPY), nitric oxide synthase (NOS), and VIP present in small quantities in the nodose ganglia were markedly upregulated in the ipsilateral nodose ganglia 14 days after axotomy (60). These changes persisted ≤42 days after nerve injury provided that axonal regeneration was not allowed, suggesting that the changes are long lasting.

Alterations in neuropeptide expression in the nodose ganglia after axotomy appear to be peptide specific. Helke and Rabchevsky (12) reported that sectioning of the cervical vagus resulted in a rapid (1 day) reduction in the number of tyrosine hydroxylase (TH)-containing cells, whereas VIP-containing neurons were dramatically increased by 3 days after vagotomy. CGRP- and substance P-containing cells in the nodose ganglia were relatively unaffected by axotomy (12). The functional significance of the changes in neuropeptide expression in the nodose ganglia after vagotomy is not known. Certain peptides such as galanin- and pituitary adenylate cyclase-activating polypeptide that showed increased expression after nerve injury may have trophic functions during nerve regeneration (49). In addition, both VIP and NPY stimulate neurite outgrowth

from dorsal root ganglia (DRG) in vitro. Hence the increased expression of some of the neuropeptides or their receptors in the nodose ganglia after vagotomy may play an important role in sensory regeneration after nerve injury. Such changes may also protect against further injury. For example, compared with CCK-A receptors, CCK-B receptors were expressed in very low levels in the vagus and the CCK gene was not expressed in the nodose ganglia. After vagotomy, there was a dramatic increase in CCK-B receptors accompanied by an increase in CCK immunoreactivity in the nodose ganglia (1). In vitro studies (23) showed that activation of CCK-B receptors may prevent glutamate-induced neuronal cell death in rat neuron cultures.

Recent studies also showed that vagotomy dramatically decreased the excitability of nodose ganglion neurons by increasing the threshold potential to >200% and reducing action potential discharge by ≤80% in response to strong depolarizing stimuli. This was mediated by reducing sodium currents after neural injury (65).

The mechanisms that trigger changes in neuropeptide expression are not completely understood. Synthesis of neuropeptides and TH are upregulated by a number of mechanisms. These include the pattern, frequency, or intensity of neuronal depolarization, retrograde transport of growth factors, and metabolic or hormonal factors (38). In the rat sciatic nerve, it was demonstrated that intermittent low-energy stimulation did not enhance regeneration after a crush lesion, whereas continuous stimulation increased nerve growth (52). Higher-energy stimulation stimulated nerve growth independent of the mode of stimulation. Other studies showed that peripheral but not central axotomy prompted axonal outgrowth and induced synthesis of NPY, galanin, and VIP in the nodose ganglion (48). These suggest that both axonal outgrowth and expression of neuropeptides in the sensory neurons could be regulated by the contact of the cells with their peripheral but not central targets.

There is increasing evidence that proteins made at the site of the lesion and then retrogradely transported to the soma may activate a gene program required for the regeneration process (22) (Fig. 1). These protein molecules may be target-derived factors that are retrogradely transported or factors derived from nontarget cells such as macrophages or Schwann cells at the lesion sites. The observation that treatment of the vagus nerve with vinblastine, an agent that inhibits axonal transport, induces upregulation of galanin and VIP (64) is consistent with a retrograde control of the expression of these peptides.

In the DRG, substance P expression appears to be regulated by target-derived nerve growth factor (NGF) (39, 43). However, NGF does not play a main role in controlling NPY and VIP expression in these cells (21). It is also unlikely that NGF plays a major role in the upregulation of galanin and VIP in the nodose ganglion because only a few cells in this ganglion express NGF receptor (21). Furthermore, NGF has little effect on axonal outgrowth from the cultured vagus nerve. Sev-
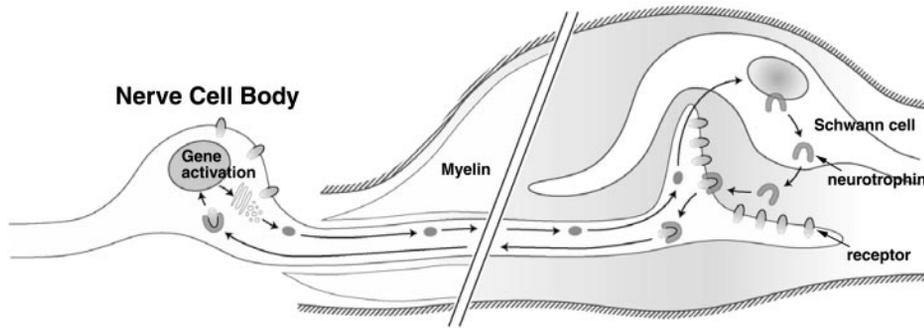
Fig. 1. After nerve injury, a signal is sent from the injured nerve to trigger the synthesis of neurotrophins in cells surrounding the axon such as the Schwann cell. The neurotrophins are secreted, picked up by receptors on the damaged axons, and retrogradely transported to the cell body, in which they activate a gene program required for the regeneration process. (Adapted from Ref. 22)

eral studies (4, 25, 65) reported that leukemia inhibitory factor (LIF), which is produced by nonneuronal cells after injury, activated the expression of galanin, substance P, and NPY in the DRG and superior cervical ganglia when retrogradely transported by a subpopulation of sensory neurons. The possibility that LIF may play a role in the upregulation of neuropeptides in axotomized neurons of the nodose ganglion should be considered.

After nerve injury, inflammatory cells such as macrophages usually migrate to the site of a peripheral nerve lesion (48). These inflammatory cells may promote nerve regeneration by inducing degeneration of the distal nerve stump, stimulating Schwann cell proliferation and inducing NGF production. It is conceivable that factors derived from these nontarget cells may contribute to the regenerative propensity and neuropeptide expression in the nodose ganglion after peripheral vagotomy.

Vagal motorneuron degeneration after axotomy. In contrast to the relatively extensive sensory reinnervation of the gut, motor fibers failed to reinnervate the gastrointestinal tract even 45 days after vagotomy (46). There are several potential mechanisms that may be responsible for the failure of the efferents to regenerate (46). Competition for limited target sites and/or the trophic factors they produce might block vagal efferent reinnervation of the target organ. Because the neurons of dorsal motor nucleus of the vagus (DMNV) are farther than the nodose neurons from the target site and regeneration rates may be slower far from the cell bodies, regrowing afferent neurites, which account for 75% of all fibers in the abdominal vagus, may reach the gut tissue earlier and effectively monopolize all viable Schwann cell sheaths in the distal vagal stump. In addition, vagal preganglionics project to the myenteric and submucous plexus that receive extensive extrinsic neural inputs and may reorganize after partial denervation. This may potentially preempt sites previously occupied by vagal preganglionic terminals and thus thwart efferent regrowth.

It is well known that degeneration of vagal motor neurons occurs after vagotomy (27, 50, 55). In fact, the DMNV neurons appear to be much more severely affected by axon injury compared with the other peripherally projecting neurons (31, 42). After axon injury, DMNV neurons atrophied and died such that after 18 mo only 25% remained. This may be due to a relative

lack of one or more requisite trophic factors from the target organ. In fact, a recent study demonstrated that the degeneration of DMNV neurons after vagal injury was significantly reduced by a bolus administration of fibroblast growth factor-1 (FGF)-1 or acidic FGF to the vagus nerve trunk immediately after a crush injury to the nerve (19). The administration of FGF-1 also enhanced axon regeneration in the vagus trunk.

The exact molecular mechanisms responsible for the induction of the death of vagal motor neurons after axotomy remain to be elucidated. With the use of in situ hybridization, RT-PCR, and immunohistochemistry, a recent study (20) demonstrated that the activation of the signal pathway for NMDA receptor 1, calcium-neuronal NOS (nNOS), and the upregulation of inducible NOS in the DMNV may be responsible for vagal neurodegeneration after vagotomy. The molecular events may involve the activation of Bcl-2, BAX, and caspase-3 to initiate the apoptosis pathway.

Role of neurotrophins in the survival and growth of injured vagus nerve. There is increasing evidence that neurotrophins such as NGF, brain-derived neutrotrophic factor (BDNF), neurotrophin (NT)-3, and NT-4/5 may play an important role in the regulation of neuropeptide phenotype expression and axonal growth. The actions of neutrophins are mediated by the low-affinity neutrotrophin receptor (P75^{NTR}) and the high-affinity receptor tyrosine kinase (Trk) family. P75^{NTR} shows similar affinities for each of the neurotrophins. On the other hand, TrkA preferentially binds NGF, whereas TrkB preferentially binds BDNF and NT-4/5, and TrkC shows a high affinity for NT-3 (5, 37). Neurons of the nodose ganglia contain primarily TrkA and TrkC receptors and transport NGF and NT-3 but not BDNF (11, 62), whereas efferent parasympathetic neurons of the vagus nerve mainly contain TrkB and TrkC receptors and transport BDNF and NT-3 (11).

In situ hybridization studies showed that axotomy of the cervical vagus nerve stimulated the expression of NGF, BDNF, and NT-3 and their receptors TrkA, -B, and -C in nonneuronal cells at both the proximal and distal segments of the transected cervical vagus nerve (59). In addition, NGF protein was increased in the distal end of the transected nerve, whereas NT-3 protein was increased in both the proximal and the distal ends of the transected nerve after axotomy. It is important to note that neurons containing neurotropin-mRNA were not found in the nodose ganglia. By 45

days after axotomy, a time when axonal reconnection with target tissue is made, the levels of neurotrophin and Trk mRNAs in the vagus nerve declined to preaxotomy levels (30). Taken together, these observations suggest an injury-induced upregulation of mRNA expression in neurotrophins and their receptors in nonneuronal elements such as the Schwann cells and macrophages. Secretion of these factors by local nonneuronal cells may be important for axonal survival and regrowth. In fact, in an in vitro study in which nodose ganglia were explanted on an extracellular matrix-based gel, it was demonstrated that NT-4, and to a lesser degree BDNF, stimulated axonal growth from the nodose ganglia (59). NT-4 acts via TrkB receptors that are present on growing nodose neurons. Activation of TrkB receptors led to mitogen-activated protein kinase.

In addition to regulation of axonal growth, neurotrophins can also alter neurotransmitter and neuropeptide phenotype expression. A recent study (13) showed that the addition of NGF to nodose ganglia culture significantly increased the number of TH-containing neurons and decreased VIP-immunoreactive neurons but did not affect the number of CGRP-containing neurons. On the other hand, NT-3 increased the number of VIP-immunoreactive neurons but did not alter the number of TH- or CGRP-immunoreactive neurons, whereas NT-4 had no significant effects on these three types of neurons. NT-3 mRNA was noted in nonneuronal cells of the vagus nerve trunk immediately proximal and distal to a nerve lesion. In vivo, this nonneuronally derived NT-3 may act on the injured neuron. The opposite actions of NT-3 and NGF on the number of VIP-immunoreactive neurons are intriguing and perhaps clinically significant. It is conceivable that in different specific clinical or therapeutic situations, NT-3 vs. NGF trophic support may be called into play after specific types of injury.

DIABETIC VAGAL NEUROPATHY AND NEUROTROPHINS

Diabetic autonomic neuropathy is accompanied by alterations in autonomic and visceral afferent neurons. Demyelination of axons of the vagus nerve occurs with diabetic neuropathy. In addition, degeneration and regeneration of unmyelinated vagal nerve fibers have also been reported in patients with diabetic gastropathy. Some of these changes may be due to hyperglycemia-induced metabolic abnormalities in the vagus nerve such as elevation in hexose and polyol levels.

Changes in the content of specific neurochemical agents such as nNOS and TH in diabetic nodose ganglion were similar to those noted after vagotomy (29, 47a). However, the changes were not as large in magnitude, as extensive, or reflected by changes in mRNA content as those noted after vagotomy. For example, in contrast to the dramatic increase in VIP immunoreactivity and mRNA in the nodose ganglion neurons after vagotomy, diabetes has no apparent effect on levels in these neurons (29). It is conceivable that either the

nature or the extent of nerve injury resulting from the diabetic state is insufficient to trigger the pronounced increases in VIP noted in vagotomy. Alternatively, diabetes may result in a reduced ability of the VIP gene to upregulate expression. Another notable difference from vagotomy is that in the nodose ganglion of diabetic rats, an increase in the number of nNOS-immunoreactive neurons occurred without an accompanying increase in nNOS mRNA, whereas both parameters were increased after vagotomy. This observation may reflect a diabetes-induced alteration in axonal transport and subsequent buildup of the nNOS enzyme in the cell body. Hence, diabetes affects the content of some but not all neuroactive agents in the nodose ganglion. This may reflect a modest level of diabetes-induced damage or alterations in axonal transport in the vagus nerve.

Neurotrophins have been shown to play an important role in the survival and maintenance of peripheral somatic and autonomic neurons. Hence, abnormal availability of the neurotrophins may contribute to the development and progression of diabetic neuropathy. In fact, decreased axonal accumulation of endogenous NGF and NT-3 in the vagus nerve of streptozotocin-induced diabetic rats has been reported (29). However, a recent study (28) demonstrated there were no changes in the NGF and NT-3 protein or mRNA levels in the stomach or atrium, two vagally innervated organs. Moreover, the amounts of neurotrophin receptors P75, TrkA, and TrkC mRNAs in the vagus nerve and nodose ganglion were not reduced in diabetic rats (28). These results suggest that neither diminished access to target-derived neurotrophins nor the loss of relevant neurotrophin receptors accounts for the diabetes-induced alteration of neurotrophins in the vagus nerve. This abnormality appears to be secondary to decreased retrograde axonal transport of the neurotrophins, which may lead to a deficit of trophic support for vagal afferent and/or efferent neurons.

The abnormal axonal transport of neurotrophins by the vagus appears to reflect alterations in basic axonal transport mechanisms in both the afferent and efferent vagus nerve (61). Axonal degeneration may contribute to the observed reduction in retrograde transport. Although some evidence exists for diabetes-induced degenerative changes in the DMNV (57), none has been reported for the nodose ganglion. Alternatively, the decreased axonal transport may be due to impairment of endocytosis or malfunctioning of the neurofilament or tubulin that are critical to axonal transport (58).

PLASTICITY OF THE ENTERIC NERVOUS SYSTEM AFTER VAGOTOMY TO MAINTAIN GASTROINTESTINAL FUNCTION

Vagotomy not only removes the vago-vagal reflex but may also trigger adaptive changes within the enteric nervous system to minimize the loss of gastrointestinal functions resulting from the interruption of the vago-vagal pathways.

Adaptive changes of the gastric intrinsic nervous system after vagotomy. Acute vagotomy results in decreased tone, dilation of the stomach, weakened peristalsis, and delayed gastric emptying. Subsequent studies, however, demonstrated an increased gastric tone, rather than a decrease after vagal section. More precisely, vagotomy impaired the reservoir function of the stomach. The adaptive relaxation of the proximal stomach in response to gastric distension was no longer observed, and therefore the intragastric pressure of the distended stomach was higher than normal (56).

The accommodation reflex allows the stomach to accommodate large volumes of food with minimal increases in pressure. Abnormal accommodation reflex occurring after acute vagotomy may result in early satiety and postprandial fullness.

Studies (56) indicate that the vago-vagal reflex plays a prominent role in mediating the gastric accommodation reflex. It involves a capsaicin-insensitive vagal afferent pathway that transmits sensory information from tension receptors located in the serosa and/or muscle layers. The vagal cholinergic efferent pathway releases nitric oxide from the gastric myenteric plexus via nicotinic receptor stimulation to mediate gastric relaxation (40). After acute vagotomy, the vago-vagal pathway is interrupted resulting in a loss of gastric accommodation reflex (Fig. 2). This is accompanied by a marked reduction of NOS in the gastric myenteric plexus (40). The synthesis and gene expression of nNOS in the gastric myenteric plexus are controlled by the vagal nerve and nicotinic synapses (40). Activation of the nicotinic receptors in the gastric myenteric neurons stimulates the phosphoinositol cascade, which in turn activates a Ca^{2+}-dependent PKC pathway to regulate nNOS expression (40). Removal of nicotinic receptor activation after vagotomy reduces nNOS expression and disrupts the gastric accommodation reflex (Fig. 3).
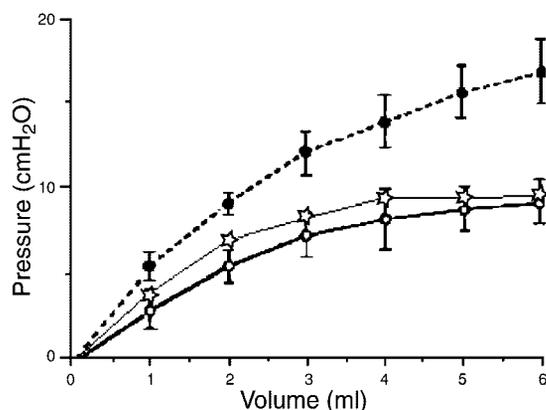

Fig. 2. In vagally intact rats (○), gastric distension caused a linear increase in intragastric pressure, but gastric distension beyond 3 ml caused a much smaller increase in intragastric pressure. Acute vagotomy (●) significantly changed the slope of the volume-pressure curve and produced a steeper increase in pressure with progressive gastric volume distension. The gastric accommodation reflex was fully restored 4 wk after vagotomy (☆) (56).

In rats, the gastric accommodation reflex was fully restored 4 wk after truncal vagotomy (56) (Fig. 2). This was not due to return of function of the vago-vagal reflex or mediation via the sympathetic pathway. With the use of a denervated, vascularly isolated, perfused stomach, it was demonstrated that gastric distension caused a smaller increase in intragastric pressure in stomach obtained from chronically vagotomized rats than that observed in stomach obtained from sham-operated rats (56). Furthermore, the pressure increase evoked by gastric distension was significantly enhanced by L-NAME, hexamethonium, and tetrodotoxin. This suggests that after chronic vagotomy, gastric accommodation is mediated by local gastric myenteric plexus involving nicotinic synapses and the intramural NO pathway. These observations support and extend the findings by Grundy et al. (8), who reported that the gastric accommodation reflex returns to normal after chronic vagotomy and that this was mediated by the intrinsic neural pathway. This may explain the observation that after chronic vagotomy, patients have a normal accommodation reflex (14).

Mechanisms responsible for the plasticity of the enteric nervous system after chronic vagotomy are not well understood. In dogs, immediately after vagotomy, the antral electrical activity showed periods of total disorganization with groups of slow potentials (electrical control activity) exhibiting various amplitudes, frequencies, and configurations (24, 45). In contrast, the electrical activity of the stomach body was not affected. As a result, the electrical control activities were no longer synchronized. The abnormal antral electrical control activities were not followed by electrical response activity, and so they did not trigger muscle contractions. The normal pattern of antral electrical activity was restored within a week, although some dogs continued to have occasional periods of disorganized rhythm for several months after vagotomy (45). Interestingly, after restoration of the normal electrical activity, perturbations similar to those observed in the early postvagotomy period can be provoked by muscarinic blockade with atropine (45). Hence electrical activity disturbance ensuing from vagotomy very likely results from a decrease in cholinergic tone, which is then able to recover, probably because of adaptive changes in the intramural cholinergic networks.

Remodeling of the neural circuitry responsible for CCK action on pancreatic secretion after chronic vagotomy. CCK is the major mediator of postprandial pancreatic exocrine secretion. Previously, we (32, 44) demonstrated that the sites of CCK action to stimulate pancreatic secretion are dose dependent. CCK doses that produce physiological levels of CCK in plasma act by stimulating the vagal afferent pathways originating from the gastroduodenal mucosa, whereas doses that produce supraphysiological CCK levels stimulate intrapancreatic neurons and pancreatic acini (32, 44) (Fig. 4).

The effect of vagotomy on pancreatic secretion remains controversial. Both profound effects (9, 32) and an absence of effects have been reported (3, 26,

Fig. 3. Proposed mechanisms of impaired nNOS expression in the gastric myenteric plexus after vagotomy. Vagal release of acetylcholine from the nerve terminal of preganglionic fibers regulates the gene expression and synthesis of nNOS in the gastric myenteric plexus through nicotinic synapses. Reduced cholinergic stimulation in the myenteric plexus after vagotomy results in decreased neuronal nitric oxide (NO) synthase (nNOS) mRNA expression, nNOS synthesis, NO production, and nonadrenergic, noncholinergic relaxation (40).
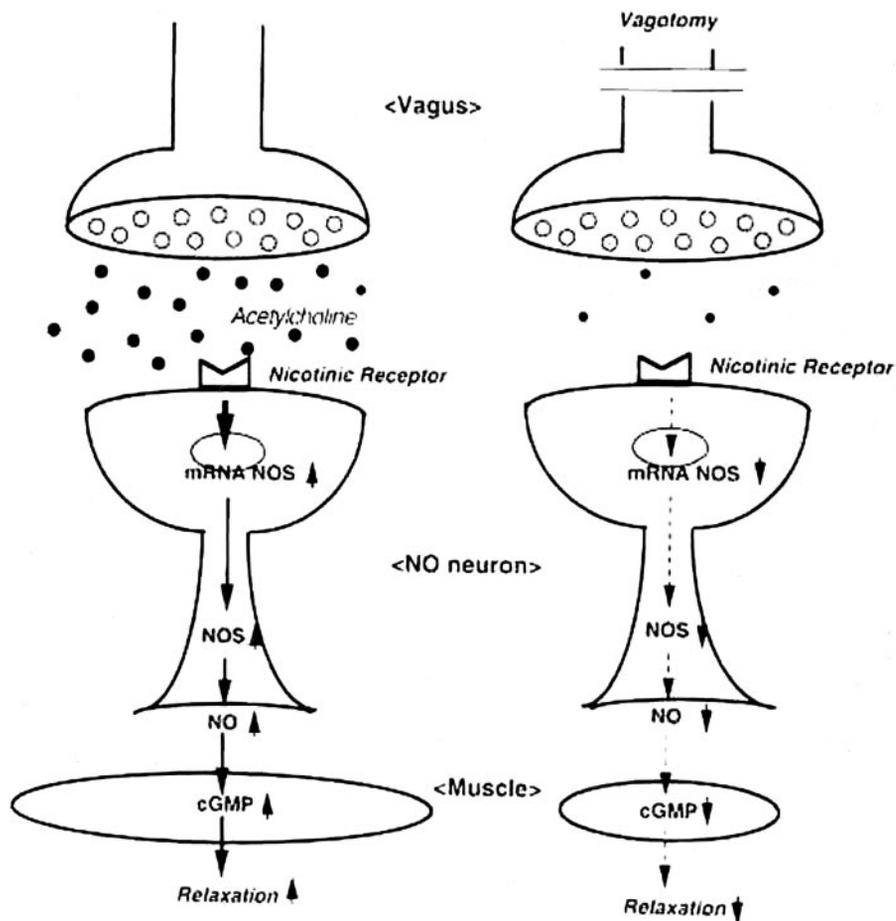
54). It is possible that the variable effects of vagotomy on pancreatic secretion may be related to the different lengths of time after surgical vagotomy and before experiments are begun. Structural and functional studies indicate the existence of an enteropancreatic pathway, although the physiological importance of this pathway remains to be elucidated. Using anesthetized and conscious rats models, we showed that after chronic vagotomy, pancreatic protein secretion in response to CCK-8 stimulation was fully restored by *day 20* (33, 44). In contrast to its effect in rats with an intact vagus nerve, atropine failed to inhibit CCK-8-stimulated pancreatic secretion in rats with a chronic vagotomy. Hexametho-

Fig. 4. *Left*: sites and mechanisms of action of CCK to stimulate pancreatic secretion. Physiological levels of plasma act via stimulation of the vagal afferent pathways. In contrast, supraphysiological plasma CCK levels act on intrapancreatic neurons and to a larger extent on pancreatic acini. *Right*: adaptive changes occur after chronic vagotomy involving recruitment of intraduodenal cholinergic neurons that activate a gastrin-releasing peptide neural pathway to stimulation secretion.
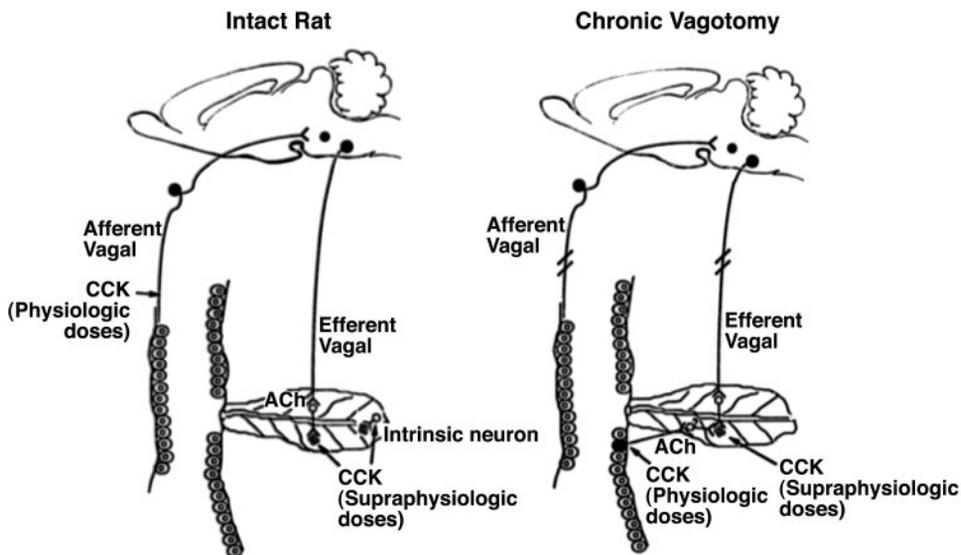
nium treatment and surgical interruption of the enteropancreatic neural connection each markedly reduced pancreatic responses to CCK-8. Moreover, application of benzalkonium chloride to the duodenal serosa ablated myenteric neurons as expected and also abolished the CCK-8-stimulated pancreatic response. Further study showed that a potent gastrin-releasing peptide (GRP) receptor antagonist markedly reduced pancreatic responses to CCK-8 in chronically vagotomized rats but had no effect in vagally intact rats (33). Immunohistochemical studies demonstrated that CCK-8 administration evoked an increased percentage of c-Fos-positive pancreatic neurons containing choline acetyltransferase in vagally intact rats; whereas the number of c-Fos-positive neurons containing GRP increased after chronic vagotomy. These observations support the hypothesis that neural remodeling occurs after chronic vagotomy and involves recruitment of intraduodenal cholinergic neurons that become responsive to CCK-8 and activate an intrapancreatic GRP neural pathway to mediate pancreatic secretion (Fig. 4).

To further investigate the adaptive changes of the enteric cholinergic neurons projecting to the pancreas after chronic vagotomy, immunocytochemical staining demonstrated that there was no difference in the number of duodenal cholinergic neurons projecting to the pancreas in duodenal specimens obtained from control and chronically vagotomized rats. Functional studies showed that the threshold dose of CCK-8 needed to stimulate [^{3}H]ACh release from the myenteric neurons in vagotomized rats was 1,000-fold less than that observed in control rats (36). Receptor binding studies showed no change in receptor numbers but a significant increase in affinity in the myenteric neurons obtained from vagotomized rats compared with controls (36). In addition, we showed that the CCK analog JMV-180, an agonist on high-affinity CCK receptors and an antagonist on low-affinity CCK receptors, did not stimulate [^{3}H]ACh release from myenteric neurons from control rats but evoked a marked increase in [^{3}H]ACh release in vagotomized rats. On the other hand, JMV-180 inhibited [^{3}H]ACh release stimulated by CCK-8 (10^9 M) in control but not in vagotomized rats (36). These observations indicate that adaptive changes occurred in the duodenal myenteric cholinergic neurons after vagotomy. This resulted in a conversion of CCK receptors from low- to high-affinity states, but the number of receptors or the number of cholinergic neurons did not increase. These changes are critical for the development of a novel enteropancreatic pathway that mediates the action of CCK after chronic vagotomy. The mechanism responsible for the upregulation of the affinity state of the CCK-A receptors remains to be elucidated.

SUMMARY

Vagal pathways appear to have an extraordinary plasticity to regain functions when challenged with neural injury. In addition, the enteric nervous system is equipped to develop compensatory changes to preserve digestive functions after vagotomy. In general, neuropeptides and transmitter-related enzymes in the sensory neurons are downregulated in response to nerve injury. This may be protective by rendering the sensory neurons less excitable. At the same time, molecules that are not present, or present only at low levels, begin to appear or become upregulated, and then play a role in the survival-regeneration process. Neurotrophins and other growth factors made at the site of the lesion and retrogradely transported to the soma may activate a gene program required for the regeneration process. Vagotomy may also trigger adaptive changes within the enteric nervous system to minimize the loss of gastrointestinal functions resulting from the interruption of vago-vagal pathways. The accommodation reflex is restored after chronic vagotomy because of adaptive changes in the intramural cholinergic networks in the stomach. Remodeling of the neural circuitry in the duodenum is responsible for the action of CCK on pancreatic secretion after chronic vagotomy. Characterization of the molecular and cellular mechanisms directing these adaptive changes in the enteric neural networks clearly requires further research.

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