Aging and Neural Control of the GI Tract
I. Age-related changes in the enteric nervous system

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Wade, Paul R. Aging and Neural Control of the GI Tract. I. Age-related changes in the enteric nervous system. Am J Physiol Gastrointest Liver Physiol 283: G489–G495, 2002; 10.1152/ajpgi.00091.2002.—As we enter the 21st century, the segment of the population that is the most rapidly expanding is that comprised of individuals 85 yr of age and older. Dysfunctions of the gastrointestinal (GI) system, including dysphagia, constipation, diarrhea, and irritable bowel syndrome are more common complaints of the elderly, yet our knowledge of the aging GI tract is incomplete. Compared with the rapid advances in the neurobiology of aging in the central nervous system, the understanding of age-related changes in the enteric nervous system (ENS) is poor. In this brief review, I recap experiments that reveal neurodegenerative changes and their functional correlates in the ENS of mice, rats, and guinea pigs. Clinical literature seems indicative of similar structural and functional age-related changes in the human ENS. Current studies that address the mechanisms underlying age-related changes in the ENS are introduced. The future directions for this field include physiological and pharmacological studies, especially at cellular and molecular levels. Research in the aging ENS is poised to make major advances, and this new knowledge will be useful for clinicians seeking to better understand and treat GI dysfunction in the elderly.

gastrointestinal tract; autonomic nervous system; degeneration; senescence

THE ENTERIC NERVOUS SYSTEM (ENS) is the division of the autonomic nervous system that is intrinsic to the gastrointestinal (GI) tract and is a principal regulator of GI function. There is extensive literature documenting the enteric neurobiology that has led to our current understanding of the form and function of the ENS as a large and complex nervous system capable of autonomously mediating reflex behaviors underlying GI motility, absorption, and secretion (14, 43). The ENS is able to mediate these reflexes independently, because the system is comprised of intrinsic sensory neurons, interneurons, and motor neurons interconnected in microcircuits. An understanding of what changes occur in the ENS with age, and how its altered state may relate to GI function in the old animal, has increasing relevance to clinical medicine. Individuals >80 yr old comprise the most rapidly growing segment of the U.S. population (33) and generally require more health care (using ∼1⁄3 of annual healthcare dollars (2)); furthermore, those >65 yr old access medical care for GI-related problems more frequently than do those in other age groups, especially for functional complaints such as constipation (8) or irritable bowel syndrome (38). On the basis of clinical evidence and demographic considerations, physicians, especially gastroenterologists, increasingly will be called on to understand and discriminate between disease processes with GI complications that are more common in the aged (e.g., diabetes mellitus), the GI side effects of multiple drugs that elderly are often prescribed, and the normally altered bowel function of the elderly. It is therefore necessary to know what “normal” is in the aged gut, and a logical starting point would be to understand the age-related changes in the ENS.

The goal of this short thematic review is to briefly summarize the enteric neurobiology of the aging gut. First, clinical studies that have addressed functional changes possibly related to altered enteric neural control are discussed. Second, the current concepts of aging in the nervous system in general are outlined as an introduction to a concise review of the structural and functional data on the aging ENS that has been accumulated to date from animal models. Finally, new understanding in this field is highlighted together with suggestions for areas where increased research efforts may lead to better understanding of the age-related processes involving the ENS that may contribute to altered physiological responses observed in the GI system of the elderly. The imposed limits in the length and number of citations means that not every work has been cited, for which I apologize in advance; nevertheless, I have directed the reader to more inclusive liter-
CLINICAL EVIDENCE OF AGE-RELATED ALTERED GI FUNCTION AND CHANGES IN THE HUMAN ENS

Hippocrates observed that in general, “...the intestines tend to become sluggish with age,” and contemporary conventional wisdom holds that gut function changes with age; however, there is less evidence-based support for this idea than one might expect. Much of the clinical literature suggests that as humans age, their GI tracts continue to function adequately despite an age-related loss of enteric neurons. The following is a concise summary that relates gut function that may occur because of intrinsic neuronal changes have yet to be determined.

AGE-RELATED CHANGES IN THE CNS AND EXTRAINTESTINAL PNS

Normal aging of the brain does not include a global loss of large numbers of neurons; rather, there are more specific, selective, and subtle changes, such as atrophy of pyramidal neurons, loss of synaptic contacts, decreases in specific neurotransmitter receptors, reactive gliosis, and abnormal cytoskeletal structures (18). Mechanisms thought to be involved in neurodegeneration of aging in the CNS that are relevant to the ENS recently have been reviewed (16). These include altered Ca$^{2+}$ signaling, mitochondrial dysfunction, the production of reactive oxygen species, and the Janus face of nitric oxide (NO; too much or too little may be deleterious). The vast literature on age-related CNS neurodegeneration is well beyond the scope of this brief article, and the changes that take place in the CNS at molecular, cellular, and functional levels have been reviewed elsewhere (20). In general, the aging mam-
malian brain shows transcriptional changes that evidence decreased protein turnover (suppression of the ubiquitin-proteasome pathway), a reduction in trophic factors [e.g., glial cell line-derived neurotrophic factor (GDNF)], and an increase in markers of inflammation and stress responses (e.g., cyclooxygenase-1, c-Fos, and heat shock factors, such as heat shock protein 40) (18). Not surprisingly, similarities between the aging CNS and the aging ENS are now coming to light. A good example of these parallels is seen in the neuron-sparing effects of caloric restriction in both nervous systems, which prevents many of the changes outlined above (Ref. 18 and see BACK TO THE FUTURE OF THE AGING ENS).

In the PNS, pre- and paravertebral sympathetic ganglia of aged humans and rodents exhibit terminal axonal dystrophy and remodeling of synapses more than neuronal cell loss (30). Because the neurodegenerative changes are seen only in some neurons, it has been suggested that certain adult postganglionic sympathetic neurons adapt phenotypes that are resistant to the neurodegeneration of aging. One of the characteristics of the aging-resistant postganglionic sympathetic neurons is that they have an increased capacity to take up trophic factors [e.g., nerve growth factor (NGF)] (6). Recent neuroanatomical tracing studies have failed to show neurodegenerative changes in motor or sensory vagal fibers that project to the gut in rats; rather, there is morphometric data demonstrating that the density and distribution of this innervation to the upper GI tract remains stable between the ages of 3 and 24 mo, and a similar qualitative assessment of the small bowel suggests that its vagal innervation likewise does not change with age (24).

NEURODEGENERATION IN THE ENS IS A CHARACTERISTIC OF AGING IN RODENTS

The general architectural form of the ENS consists of two ganglionicplexuses, the myenteric plexus situated between layers of the muscularis externa, and the submucosal plexus in the connective tissue between the inner circular layer of smooth muscle and the muscularis mucosae (14). This plan is relatively constant throughout the length of the gut and similar across mammalian species, although there are regional differences in the population densities of enteric neurons and in the phenotypes of these neurons as well as interspecies differences in the ENS. It is, therefore, important to assess what age-related changes take place along the entire GI tract and to learn whether these changes occur in several species. This section summarizes the evidence for an enteric neurodegeneration of aging that has been accumulated from three commonly studied species: guinea pig, rat, and mouse. The question that may arise is how old is “old” in a laboratory-raised rodent? Although the answer to this fundamental question is beyond the scope of this review, laboratory-raised mice may live as long as 4–5 yr, rats as long as 5–6 yr (12), and guinea pigs as long as 7–8 yr (M. D. Gershon, personal communication).

GUINEA PIG

This species has been widely used in enteric neurobiology and was one of the first animals in which an age-related decrease in the total population of neurons was noted. Nevertheless, there is a paucity of evidence concerning regional changes in the ENS that occur over time. Gabella (13) studied whole mount preparations of small intestine consisting of longitudinal muscle and myenteric plexus (LMMP) stained with nitro blue tetrazolium (NBT) for β-NADH reductase and determined that the population of neurons decreased by approximately one-half between the ages of 3 and 30 mo. Recently, we have been using immunocytochemistry for RNA binding proteins (Hu C/D) ubiquitously expressed in all vertebrate neurons to estimate enteric neuronal populations, and our preliminary evidence is that a significant neuronal loss (~18%) occurs in the submucosal plexus in guinea pig distal colon between 5 and 20 mo and before neurodegeneration of the myenteric plexus can be detected (by 27 mo, neuronal loss in the myenteric plexus was ~40% (40)). Evidently, no other comparable studies have been completed in regions of the guinea pig gut outside the small intestine and distal colon.

RAT

The ENS of the rat has been extensively studied, and rats have also been used in a number of studies of the age-related changes in the ENS. One of the first studies (29) was based on the NBT NADH staining (described above) of jejunal, ileal, colonic, and rectal LMMPs and revealed a loss of ~40% of myenteric neurons between the ages of 6 and 24 mo in small bowel and up to 60% neuronal loss in the colon. Powley’s group (24) recently used cuprolinic blue-stained LMMP whole mounts to estimate changes in myenteric neuron populations throughout the rat GI tract at five time points between 3 and 27 mo. This elegant, comprehensive study revealed no age-related loss of myenteric neurons in the gastric corpus or antrum but showed decreases of 20–30% in the duodenum, 15–20% in the jejunum, and 30–40% in the colon and rectum. Many investigations on age-related changes in the ENS typically have been based on only two ages; however, Phillips and Powley (24), by virtue of employing multiple time points, were able to calculate a rate of neuron loss for each organ of the rat GI tract and showed that there is neuronal loss as early as 12 mo of age and that the neurodegeneration continues in an approximately linear fashion throughout the remainder of the animal’s life.

MOUSE

Comparable studies based on whole mount preparations of mouse gut apparently have not been carried out; however, point-counting stereological analyses of histological sections of mouse gut, immunostained for the neuronally expressed protein gene product 9.5, have been reported (9). The number of submucosal and
myenteric ganglia remains constant in the gastric antrum, duodenum, and distal colon of mice between 1 and 24 mo; however, the number of neurons per ganglion declines by ~30% in the antrum and by 50–60% in the duodenum and distal colon between 3 and 12 mo of age. Notably, the population both of submucosal and myenteric neurons is apparently stable between 12 and 24 mo of age in mice. It is surprising, given the relative ease of manipulating the mouse genome, that more studies have not been done establishing a mouse model for the aging ENS.

Thus, by a variety of approaches, the phenomenon of age-related neurodegeneration of the ENS has been established in three rodent species commonly used in enteric neurobiology. Beyond the general observation that significant numbers of neurons in the ENS are lost as these animals age, comparatively little else is known. To understand the changes in GI function that are observed in advanced age, it will be necessary to learn the fate of specific neuronal phenotypes within the aging ENS.

EVIDENCE OF AGE-RELATED CHANGES IN SPECIFIC PHENOTYPES OF ENTERIC NEURONS

The earliest descriptions of the ENS in rodents of advanced age showed marked loss of neurons (see above), but the investigators also wanted to know the characteristics of neurons that were lost. On the basis solely of morphological properties of surviving neurons, Gabella (13) determined that especially large neurons in the guinea pig small bowel myenteric plexus were lost in advanced age. Through combinations of electrophysiology, nerve tracing, and immunocytochemistry, distinct classes of neurons are now recognizable in the guinea pig (3), and with the use of this large database, it should be quite possible to learn about age-related changes of specific component neurons in the ENS of this species; however, few such studies have been reported. Calbindin is a calcium-binding protein that has been used as a marker of intrinsic sensory neurons in the guinea pig ENS (3), and our preliminary evidence is that the age-related loss of calbindin-immunoreactive (IR) neurons in the submucosal plexus of the guinea pig distal colon is greater than that of the total population of submucosal neurons (40). Furthermore, the amount of lipofuscin seems to accumulate with age in the surviving calbindin-IR neurons to a greater extent than in other submucosal neurons (40). Lipofuscin, also known as "age pigment," is a fluorescent substance (excitation maximum ~440 nm, emission maximum ~600 nm) that accumulates in lysosomes and is thought to be an indicator of oxidative stress as well as of aging (17). It is interesting to note that although lipofuscin is also present in many neurons of the aged rat ENS, it has recently been demonstrated to be absent in the neurocalcin-IR neurons of aged rats (5). Additional new data suggest that the relative size of the subpopulation of serotonin-IR myenteric neurons in guinea pig distal colon is the same in young as in old animals (40).

Brookes (3) distinguishes 18 classes of enteric neuron in the guinea pig small intestine, and there are likely comparable classes in other regions of this animal's GI tract. Many more studies will need to be completed before there is a clear understanding of the effects of aging on the various classes of neuron present throughout the guinea pig ENS.

When Santer and Baker (29) initially studied the age-related changes in the rat ENS, they observed that the neurons lost over time were of all sizes and shapes, suggesting that the neurodegeneration of aging was not limited to a particular class of neuron. When immunocytochemistry began to be employed in similar studies, other investigations suggested that different phenotypes of enteric neuron in the rat may not be equally susceptible to, or protected from, the neurodegeneration of aging. In fact, one of the earliest immunocytochemical studies illustrated a decline in the density of substance P (SP), vasoactive intestinal polypeptide (VIP), and somatostatin (Som)-IR fibers with age in the rat small bowel ENS, primarily in very old age (24–36 mo) (10). Because only neuronal processes were specifically counted and not cell bodies, one is left to infer from this study that sensory neurons (SP-IR), inhibitory motor neurons (VIP-IR), and interneurons (Som) are affected by the aging process in rats.

In addition to VIP, NO is an important inhibitory neurotransmitter synthesized and released by a subset of neurons in the ENS (14). In these enteric neurons, NO is generated as a byproduct of the l-arginine conversion to l-citrulline that is catalyzed by the enzyme NO synthase (NOS). There are three main isoforms of NOS, and enteric nitrergic neurons constitutively express a Ca²⁺-dependent isozyme [neuronal NOS (nNOS)]. Nitrergic neurons can be immunostained with antibodies to nNOS or they can be histochemically stained by the NADPH-diaphorase reaction (1). NADPH-diaphorase histochemical studies revealed a 15% decrease in the population of nitrergic myenteric neurons between the ages of 4 and 24 mo in the small bowel of rats (28) [compared with a 40% decrease in all myenteric neurons (29)], suggesting that nitrergic neurons are spared during the aging process. Even more striking is the finding that ~100% of myenteric neurons in the rat proximal colon were positively stained for NADPH-diaphorase at 26 mo of age (1). Recently, Takahashi et al. (36) obtained conflicting data by immunostaining LMMPs of rat colon with antibodies raised against nNOS; they determined that the number of nNOS-IR neurons per ganglion decreased >50% between 4 and 28 mo.

As additional information comes to light, it seems increasingly evident that not all enteric neurons necessarily suffer the onslaught of aging in the same fashion; some classes of neurons may decline sooner, others later, and some may increase—with changes being regionally specific. There is clearly a need for a more complete understanding of the age-related changes within subpopulations of enteric neurons. Additionally, there is a void in the literature on age-related changes that occur in the upper GI tract of...
commonly studied mammalian species, and few studies have focused on the specialized sphincteric regions of the gut.

EVIDENCE FROM PHYSIOLOGICAL STUDIES IN ANIMALS SUGGESTS AGE-RELATED CHANGES IN ENS-MEDIATED FUNCTION

This article began with a summary of the functional changes in the aging human GI tract that may have a basis in age-related changes in the ENS. With the morphology of the aging ENS summarized both for human and laboratory animal studies, a logical question to ask is: What evidence is there that GI function changes as these laboratory animals age? This evidence is organized here according to studies aimed at understanding age-related changes in either GI motility or mucusal function.

MOTILITY

Despite the fact that the ENS is equipped with a large functional reserve of neurons, it would be surprising if neurodegeneration in the range of 40–60% of the entire population did not lead to altered physiological states of the bowel. There is evidence of functional motility changes both in the upper and lower GI tract of aged animals. The rate of gastric emptying in rats remains the same between 3 and 12 mo (31); but whether gastric emptying is slower in rats of advanced age is controversial, with one study demonstrating an insignificant decrease between 5 and 28 mo (21) and another showing a 50% decrease between the ages of 4–8 vs. 22–28 mo, and the smaller decrease was observed in the study in which young included rats up to 12 mo old. We have observed that the rate of propulsion of a solid pellet in isolated Fischer 344 rat distal colon is reduced by ~20% with age (4–7 vs. 24–27 mo.) (39).

A reduction in the number of myenteric neurons suggests that there may be a reduction in the releasable pool of neurotransmitter substances as well as fewer neurons to respond to signals. The maximal twitch response of rat colonic preparations to ACh decreases by 50% with age (27). Interestingly, the release of ACh in response to EFS is due to a reduction in the activity of neuronal w-conotoxin-sensitive (N type) Ca<sup>2+</sup> channels and not to a reduced ability of neurons in tissues from aged rats to synthesize ACh. In fact, ACh release could be restored to levels seen in tissues from younger animals by the addition of the Ca<sup>2+</sup> ionophore ionomycin (27). Molecular approaches to age-related changes in the ENS should provide answers to many questions concerning the function of enteric neurons that survive into old age. Northern blot analyses have revealed that mRNA-encoding nNOS is downregulated by as much as 70% in old (22–28 mo) rats, and immunoblots reveal an estimated reduction of nNOS protein by as much as 50% (36). In the same study, both the basal and veratridine-stimulated activity of nNOS (as measured by production of L-citrulline) was reduced by ~50% with age. Notably, ACh and NO are only two of a dozen or more neuroactive substances released by enteric neurons. Because few studies aimed at understanding age-related changes in myenteric neuronal function have been carried out, and apparently none using mice, there is clearly a need for further research.

MUCOSAL FUNCTION

Evidence of age-related neurodegenerative changes in the submucosal plexus exists (9, 40), but few studies have focused on changes in mucosal function that occur with age. Powell and Reddix (26) have recently observed that the spontaneous, neurally mediated secretory activity recorded in colonic tissues from young (3–4 mo) guinea pigs is absent in the same preparations derived from mature (12–15 mo) guinea pigs. Furthermore, the colonic tissues from the mature animals mount diminished secretory responses both to EFS and to nicotinic agonists when compared with the preparations from young guinea pigs. Because there was no age-related difference in the epithelial secretory response of these preparations to muscarinic stimulation, it seems that it is the submucosal component of the ENS, which regulates absorption and secretion across the mucosa, that is functionally altered in an age-related process.
BACK TO THE FUTURE OF THE AGING ENS

The state of the science in the neurobiology of the aging ENS is on the cusp of moving beyond recognizing certain phenomena, such as the loss of enteric neurons and altered motility or secretory states, to addressing the mechanisms by which neuronal loss and/or dysfunction in surviving neurons occurs. Organizers of a workshop on the aging GI tract convened in the late 1980s and concluded that to properly understand how the neuromuscular apparatus of the bowel changed with age, certain key pieces of information needed to be known with respect to the ENS (34), e.g., do certain types of neurons disappear in the ENS as animals age? Are there age-related changes in neuronal membrane Ca\(^{2+}\) channels or in intracellular Ca\(^{2+}\) metabolism? Does the expression of receptors change with age? And what happens to the levels of specific neurotransmitters? As outlined above, researchers have now begun to answer many of these questions. There is evidence that specific neuronal phenotypes may be more vulnerable to the aging process than others (28, 36, 39, 40), that neuronal N-type Ca\(^{2+}\) channels may be functionally impaired in the ENS (27), that there may be a decline in the ability of inhibitory motor neurons to synthesize NO (36), and that there may be a decrease in the expression or function of submucosal nicotinic receptors (26). There are clearly many unanswered questions, and these studies exemplify the future directions in which this field can rapidly advance.

Future research in the aging of the ENS will continue to move beyond phenomenology and into the realm of age-related functional changes and alteration in the underlying mechanisms. Information gleaned from work on the aging CNS and new technologies will continue to aid in the advance of understanding the aging ENS. Novel techniques such as the use of oligonucleotide microarrays, which have been used to profile gene expression in the aging CNS (18), could be used to identify age-related changes in gene expression in the ENS. Caloric restriction (CR) has been shown to radically alter the profile of gene expression in the CNS of old mice compared with age-matched animals fed ad libitum, downregulating the genes associated with inflammation and with stress responses normally seen in the aging CNS and upregulating those associated with neurotrophic factors (18). From this line of CNS research, CR has been demonstrated to reduce the neurodegeneration of aging in the myenteric plexus of rats (7) and in the submucosal plexus of mice (R. A. Reddix, personal communication). The neurotrophic factors, neurotrophin 3 (NT3) (32) and GDNF (R. A. Reddix, personal communication) are factors that seem to provide protection from neurodegeneration in the ENS and, not coincidentally, have been used to decrease colonic transit time in patients with constipation (4). Electrophysiological studies have not been carried out on the aging ENS, so it is unknown at this time whether there are remarkable differences in the membrane properties or synaptic behavior of enteric neurons in old animals. Contemporary molecular techniques should also aid in the identification of altered neurotransmitter and receptor expression in the aging ENS.

Aging, by definition, clearly occurs in the ENS, but this remarkable nervous system of the gut seems to age rather gracefully; even a 36-mo-old rat continues to eat, digest, and eliminate waste. This is not to say that the ENS of the very old animal, including human, is not normally altered with age; quite the contrary, evidence suggests that it is morphologically and functionally changed. Nevertheless, additional detailed knowledge of the aging process in the ENS is needed and should be useful in the development of effective medical therapy for common GI disorders that are encountered more frequently in the elderly.

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REFERENCES