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Aging and Gastrointestinal Neuromuscular Function: Insights from Within and Outside the Gut

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Keywords

GI motility; visceral sensation; CNS; enteric nervous system; vagus nerve; spinal ganglia; autonomic nervous system; GI smooth muscle

Introduction

This review will examine the effects of advanced physiological aging on gastrointestinal (GI) neuromuscular anatomy and function. We will emphasize contributions from the past several years, and build-upon the excellent reviews of Camilleri et al. (2008) and Bitar and Patil (2004). 1›2 We will not include references to early developmental changes in the enteric nervous system.

We will begin with a review of our current understanding of the effects of advanced age in the human GI tract and use these observations as a foundation to examine potentially relevant mechanisms underlying physiological aging. Using data from animal models, we have included references to mechanistically-driven studies that focus on extraintestinal sites where we consider that these observations have relevance to physiological aging involving GI neuromuscular function.

Effect of Aging on GI Neuromuscular Function in the Human

Background

In the absence of systemic diseases that are often associated with the use of medications that can affect the brain-gut axis, clinically-significant changes in the brain-gut axis are typically not apparent prior to the seventh decade of life.3 Alterations in taste and smell, gastric motility, increased presence in bacterial intestinal overgrowth and changes in GI hormone and neurotransmitter levels may contribute to physiological anorexia observed in advanced age. Alterations in swallowing leading to silent aspiration, as well as changes in gastric emptying may contribute to postprandial hypotension and maldigestion. Age-related alterations in the structure and function of the pelvic floor and anorectum may be a major contributor to the constipation and fecal incontinence encountered in the elderly, and age-associated weakening of the colonic muscular wall may contribute to the development of diverticulosis.4 Finally, the clinical impact of co-morbid conditions such as cardiovascular disease, hypertension and diabetes mellitus, and their treatment are likely to have a significant effect on GI neuromuscular function but this issue remains undefined.5

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Motor and Sensory Changes in the Oropharynx

Dysphagia and anorexia are common complaints in the elderly which may be in part related to the changes in motor and sensory function of the oropharynx. Aging has been reported to affect pharyngeal persistalsis, upper esophageal sphincter opening, suprahyoid muscle contraction, and deglutitive excursion of the larynx and hyoid bone which are believed to be contributors to the alteration in bolus kinematics and dynamics of pharyngeal swallowing in the elderly.6 Dejeager and colleagues demonstrated that a reduction in the driving force of the tongue, decrease in amplitude of the pharyngeal wall contraction and reduction in pharyngeal swallowing occur in the elderly contributing to the retention of food in the valleculae and piriform sinuses.7 A decrease in the afferent arm of the laryngo-upper esophageal sphincter reflex has been demonstrated to occur with age, which may also be a contributor to development of dysphagia.8 The gag reflex has been reported to be absent in 40% of healthy elderly adults.9 It has been proposed that the deterioration in sensations of taste, smell and vision seen in the elderly be a factor to the common complaint of anorexia reported in this age group.10

Motor and Sensory Changes in the Esophagus

Similarly, the changes in esophageal motor and sensory function may contribute to the dysphagia, reflex symptoms and anorexia commonly reported by the elderly. Earlier studies have consistently demonstrated a reduction in esophageal peristalsis and increase in non-propulsive contractions, and to lesser degree, a reduction in lower esophageal sphincter (LES) pressure.11, 12 A recent study by Gregersen and colleagues using manometric swallow analysis with a distention method demonstrated decreased compliance of the esophagus with age as well as a deterioration of the active contractile properties of the esophagus.13 This study also reaffirmed a reduction in primary as well as secondary esophageal peristalsis with age. Aged-related increases in esophageal motor abnormalities appears to have a significant clinical impact based on an review of 452 clinical manometric studies performed at an Australia tertiary care center.14 Similarly, a large retrospective study of patients referred for reflux symptoms demonstrated an age related decrease in LES length and esophageal motility.15 There is also evidence suggesting that the aged esophagus is less likely to sense acid reflux. 15

Motor and Sensory Changes in the Stomach

The available literature is inconsistent regarding the effects of aging on gastric motor function. Several small studies have reported conflicting data on gastric emptying times.11,12,16 The largest study to date involving 172 healthy patients demonstrated a trend towards increased gastric emptying by an average of 6 minutes for every 10 years of increase in age.17 Other observed gastric motor changes with aging include a reduction in post-prandial peristalsis and gastric contractile force.18 However, whether these motor changes have any role in the increased prevalence of anorexia and dyspeptic symptoms reported in the elderly remains to be determined. There are no reported gastric sensory changes associated with aging.

Motor and Sensory Changes in the Small Bowel

The sensory and motor function of the small bowel appear well preserved aside from minor manometric effects reported to include decreased frequency of post prandial contraction, the migrating motor complex (MMC) and propagated clustered contractions.5 In the small bowel the overall motility patterns and transit time have been reported as unchanged from those of younger adults.12,19 However, a recent multicenter trial of 172 healthy adults using a wireless motility capsule reported a statistically significant decrease in small bowel transit time with aging by an average of 12 minutes for every 10 years of increase in age.17

Although similar findings have been reported by Graff and colleagues.20 an explanation for this finding remains unclear but may represent a phenomenon related to increased prevalence of small bowel bacterial overgrowth in the elderly.

Motor and Sensory Changes in the Colon

Although the incidence of constipation increases considerably with aging, the effects of aging on colonic motility and colonic transit time appear less clear. Some earlier studies report a prolongation in colon transit time with aging, whereas more recent studies observed no change in transit time with aging.12،17،21 A reduction in the propulsive efficacy has been reported; however, this does not appear to translate into any clinically significant outcome.11،22 There are no reported aged-related changes in the sensation of the human colon excluding the rectum which is reviewed below.

Motor and Sensory Changes in the Anorectum

Much like the proximal GI tract, aging has a considerable effect on the motor and sensory function of the anorectum. Although there are studies with discrepant findings, the preponderance of published studies report age-related reduction in basal and maximum anal sphincter tone, decreased compliance of the rectal vault and increased perineal descent.5،12،19،22 These age-related changes are more pronounced in women compared to men and there is speculation that vaginal childbirth may play a role in this observation. Aging is also associated with altered rectal sensation. Most studies demonstrate rectal hypersensitivity but other studies observed reduced rectal sensation.5،12،19،22 These alterations in anorectal function are generally thought to contribute significantly to the constipation and fecal incontinence reported in the elderly. What remains less clear is whether these changes in anorectal function are a result of the aging process itself or due to other factors such as trauma related to child delivery or other co-morbidities associated with neurodegenerative effects involving the pelvic floor.21

In summary, the age related changes in motor and sensory function of the human gut appear most pronounced and best defined in the proximal and distal portions of the GI tract. This may be result of the greater role of skeletal muscle in the function in these regions of the GI tract. This may also be due to the relative ease in assessing motor and sensory of the proximal and distal GI tract in comparison to other areas the gut. A variety of changes have been reported in other areas of the GI tract. The inconsistencies regarding some age-related changes in GI motor function likely reflect differences in experimental design regarding patient selection and the methods used to assess motor and sensory function.

Aging and GI Extrinsic and Intrinsic Neural Pathways

There are numerous animal studies showing that aging has significant effects on nerves at both a supraspinal and peripheral level. Advanced age is associated with reduced neurotransmitter content and expression and there is a substantial loss of neurons and dendritic connections throughout the cerebral cortex, midbrain and brainstem.23 Aging has also been found to significantly affect the spinal cord and age-related changes have been measured in neurotransmitter content and receptor expression within the spinal cord. Specifically, it has been shown that spinal levels of calcitonin gene related peptide (CGRP) and Substance P (SP) are decreased in aged rats compared to younger animals, as is the expression of dopamine and serotonin receptors.24،25 In contrast to the paucity of information on the effect of aging on the extrinsic control of the GI tract, more information is available regarding the effect of aging on the enteric nervous system though most of this data has been obtain from rodents. Age-associated decrease in the number of enteric neurons has been implicated in several studies. For example, Phillips et al. (2003) found that neuron

loss occurs in the myenteric plexus of the aged rat.26 Specifically, age-related cell loss in the small and large intestines occurred exclusively in the cholinergic subpopulation but based on the presence of somatic hypertrophy and swollen axons that nitrergic neurons were not completely spared from the effects of age.

Kasparek et al. (2009) studied age-related changes in non-adrenergic, non-cholinergic (NANC) VIP and substance P function in the jejunum of young (age 3 months) and middle age (age 15 months) Lewis rats. In the jejunum of middle-aged rats, participation of VIP in functional NANC innervation was increased, while functional innervation with Sub P was decreased supporting age-associated plasticity in neuromuscular bowel function.27 Peck et al. (2009) examined the guinea-pig colon for age-associated changes in gut dimensions, myenteric neuron size, density, ganglionic area and motor nerve fibers in the circular muscle layer in the guinea pig.28 The density of myenteric neurons in mid-colon decreased by 50% from 2 weeks to 2 years, but when the increase in colon dimensions was considered, the number of neurons decreased by ~20%. Finally, the percentage area of motor nerve fibers in the circular muscle decreased with no change in total volume of nerve fibers. Thus, advanced age appeared to be associated with a fall in density but not the number of myenteric neurons and circular muscle fibers.

There is limited data examining the effect of aging on the innervation of the human GI tract. Hanani et al. (2004) investigated the effect of aging on the structure of myenteric ganglia in the human colon in patients aged 10 days to 91 years.29 Myenteric ganglia were classified into three types: normal, those containing empty spaces ('cavities') and those containing large nerve fiber bundles. Aging was associated with a significant increase in the proportion of ganglia with cavities. Conversely, there was a decrease with age in the proportion of normal ganglia and the proportion of fiber-containing ganglia did not change with age. Therefore, advanced age was associated with an increase in the number of abnormal appearing myenteric ganglia in the human colon. Bernard et al. (2009) studied age-associated (age 33–99 years old) changes in the number, subtypes of enteric neurons and neuronal volumes in normal human descending and sigmoid colon.30 Neuronal markers included HuC/D, choline acetyltransferase (ChAT), neuronal nitric oxide synthase (nNOS) and protein gene product 9.5. The number of neurons in the colon declined with age, although nNOS-positive neurons were spared. Of interest, these alterations were not accompanied by changes in total volume of neuronal structures supporting the presence of compensatory changes in the remaining neural population. At the peripheral level, loss in myelinated and unmyelinated nerve fibers, axonal atrophy and abnormalities in nerve conduction have been reported.31 In summary, available data suggests that advanced age likely has differential effects on subpopulations of neurons in the enteric nervous system which demonstrate regional- and species-specific differences.

Age-Related Changes in the Neural Control of the GI Musculature

Some neurological disorders can be associated with severe constipation and provide insight regarding potential mechanisms underlying age-associated neurodegeneration in the gut. For example, constipation is common complaint in patients with Parkinson’s disease (PD).32 Although PD is a progressive neurodegenerative disorder of dopamine-containing neurons in the substantia nigra, there is evidence to suggest that neuronal structures outside the brain are affected by the neurodegenerative process of PD. Recent reports using colonic biopsies from patients with PD have shown that ENS lesions are apparent. Interestingly, these studies show that the ENS abnormalities are observed during early stages of the disease and may even precede the onset of neuronal damage in the brain.33,34 Supporting these clinical observations, recent data has been obtained in rodent models of PD that have been instrumental in increasing our understanding of the changes in the neural innervation of the gut associated with PD. For example, in models of PD induced by the prototypical

Parkinsonium neurotoxin MPTP (1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine), rotenone or unilateral 6-hydroxydopamine lesions of the nigrostriatal dopaminergic neurons, the loss of enteric dopaminergic neurons is associated with abnormal colonic contractility leading to a marked inhibition of propulsive GI motility and daily fecal pellet output.35,36 Although the loss of dopaminergic neurons in the ENS may be one of the causes of constipation in PD, the presynaptic protein α-synuclein, a major component of Lewy bodies, has also been implicated in changes in the ENS in PD. Supporting the hypothesis that constipation in PD is related to abnormal levels of α-synuclein in the gut, experiments in mice over-expressing human wild-type α-synuclein, demonstrate abnormal patterns of colonic motility including increased time to expel an intracolonic bead compared to wild type mice.37 Although there is increasing scientific and clinical evidence to support a link between PD and constipation, there are no data on whether other neurodegenerative diseases that affect central neural structures also alter the innervation of the gut.

It is noteworthy that accumulation of α-synuclein immunopositive aggregates has also been reported in the myenteric plexus of the aged rat independent of PD.38 Alpha-synuclein is abundant throughout the myenteric plexus but appears to be a protein which is prone to fibrillization and, therefore, a candidate marker for age-related axonopathies. Alpha-synuclein-positive dystrophic axons and terminals were present throughout the GI tract of middle-aged and aged rats. However, other dystrophic neurites were not co-reactive for α-synuclein. In addition, a subpopulation of α-synuclein inclusions contained deposits that immunostained with an anti-tau phospho-specific antibody, but not all of these hyperphosphorylated tau-positive aggregates co-localized with alpha-synuclein. Therefore, the presence of heteroplastic and potentially degenerating neural elements and protein aggregates both positive and negative for alpha-synuclein suggest a complex chronological relationship between the onset of degenerative changes and the accumulation of misfolded proteins in the myenteric plexus.

The observation that cholinergic neurons innervating the gut are markedly altered in aged animals points to a possible enteric neurodegeneration that may mirror certain aspects of Alzheimer's disease observed in the central nervous system.39 However, a link between the overt neurodegeneration associated with Alzheimer's disease and enteric neuronal degeneration is lacking in part due to the paucity of relevant animal models and clear clinical evidence from patients with Alzheimer's disease.

Age-related Changes in the Submucus Plexus

The submucus plexus (SMP) innervating the GI mucosa is also likely to be affected in advanced age. The SMP of the large intestine plays important roles in secretion and motility, functions that are compromised in the aged. Phillips et al. (2007) examined the effects of aging on intrinsic SMP neurons and their extrinsic inputs in the colon of male Fischer 344 rats ranging in age from 6 to 27 months.40 Tissues were processed to visualize neurons and nerve fibers immunoreactive for either tyrosine hydroxylase (TH-IR) or calcitonin gene-related peptide (CGRP-IR). Significant age-related loss of SMP neurons occurred as early as 12 months of age and progressed in a roughly linear manner with age, decreasing by 38% in the distal colon. In the oldest rats, the colonic SMP routinely contained markedly swollen TH-IR axons and terminals and exhibited a reduction of ganglionic neuropil, whereas CGRP-IR fibers demonstrated only modest axonopathies. These observations support selective neural deterioration in the SMP and its extrinsic inputs.

Aging and Visceral Sensitivity

Complaints consistent with irritable bowel syndrome (IBS) including abdominal pain and abnormal bowel habits are common in the elderly.41 Although it is documented that somatic

pain sensitivity changes with advancing age, the effect of aging on visceral pain of GI origin is a largely neglected area. In one study a decrease in esophageal pain perception to graded intraesophageal balloon distension was observed in older (mean age 72.5 years) individuals compared to younger controls.42 Moreover, Lagier and co-workers (1999) found that rectal sensitivity thresholds induced by distension were increased in aged healthy human volunteers.43 However, in the same study colorectal smooth muscle compliance and tone were unaffected by aging. The underlying mechanisms responsible for this impairment of visceral perception in older people remain unknown. However, it is plausible that visceral afferents, including nociceptors and nociceptive pathways are vulnerable to age-related changes or alternatively that abnormalities in the central processing of visceral information may be the culprit. To explore the mechanism(s) underlying the age-related changes in pain, animal studies suggest that thermal pain, which is Aδ dependent, is very susceptible to aging whereas C fiber sensory function is relatively unaffected by aging.44 Furthermore, in senescence accelerated mouse models there were significant differences in nociceptive sensitivities in a battery of somatic pain tests.45 The consequences of a reduction in visceral pain perception due to aging on health care delivery has been reviewed by Moore and Clinch (2004) and include delayed diagnosis, more severe complications and longer hospital stays.46

Aging and Interstitial Cells of Cajal

Together with enteric nerves and smooth muscle cells, interstitial cells of Cajal (ICC) play a pivotal role as the “pacemakers” in the control of gastrointestinal motility. However, little is known about the effect of aging on ICC. Gomez-Pinilla et al. (2010) examined the effect of aging on ICC number and volume in the human stomach and colon.47 In both stomach and colon, the number of ICC bodies and volume significantly decreased with age at a rate of 13% per decade. ICC size was only affected in the myenteric plexus in the colon. The changes associated with age were not differentially affected by sex or colonic region. This decrease in ICC may reduce the functional capacity of the gastrointestinal motor apparatus and contribute to changes in gastrointestinal motility with aging, as well as, influence intestinal response to disease, operative interventions and medications in older patients.

Aging and Intestinal Glial Cells

Glial cells which support the myenteric plexus are also likely affected by aging.48 Significant reductions in both the numbers of glia and neurons occurred in every region of the small and large intestine sampled from aged rats, except for the rectum, where a non-significant decrease was observed. Glial loss was proportional to neuronal death, supporting an interdependency between the two cell types.

Neurogenesis and Neuroprotection in the GI Tract

The ENS has been shown to retain neural crest stem cells in the adult gut.49 However, the postnatal gut stem cells demonstrate reduced neuronal subtype potential. The first demonstration of enteric neurogenesis came from recent work by Gershon and colleagues who observed that $5HT_4$ receptor agonists promote enteric neural development and even neuronal cell survival. Furthermore, the age-dependent slowing of colonic transit is more marked in $5HT_4$ −/− mice suggesting that 5-HT may have a neural protective role in the mouse colon.50

Aging and GI Smooth Muscle

The role of smooth muscle in the wall of hollow organs of the GI tract is to maintain organ dimensions and force development. Both functions contribute to the role of smooth muscle

in modulating the “reservoir” capacity and “propulsion” of food along the length of the GI tract. Smooth muscle cell (SMC) functionality is regulated by neurogenic and myogenic components. Age-dependent changes are observed both in the cholinergic neurotransmission as well as the response of smooth muscle to acetylcholine (Ach). Age-dependent decrease in agonist-induced contraction is attributed to impairment in signal transduction pathway(s) in GI smooth muscle.

Effect of aging on mechanisms of contraction of circular smooth muscle of the gut

Among the various biological functions affected by aging is the control of motility of the gut. Colonic motility regulated by the smooth muscle contraction and relaxation cycle, declines with aging.[51,52] Colon tissue from aged mice (24–33 months) showed very rapid and prolonged VIP-induced relaxation with slow recovery to baseline force. Furthermore, aged mice also exhibited delayed and reduced magnitude of Ach-induced force generation. Altered contractile and relaxation response in muscle preparations from aged rodents is due to impairment in regulation of smooth muscle contraction/relaxation cycle. Studies have shown that circular smooth muscle preparations from aged rats had reduced translocation of protein kinase C (PKC)α and RhoA (member of the Rho family of GTPases) in response to contractile agonist. Translocation, docking and activation of PKCα is affected in aging[53] though the expression of PKCα is not. Therefore, a crucial mechanism involved in PKC translocation and activation (phosphorylation) is disrupted in aging. A “positive loop” involving heat shock protein (HSP)27 is an important mechanism involved in PKCα translocation and contraction that is affected in aging. Reduced expression of caveolin (cav)-1 is observed in aging resulting in reduced translocation and docking of PKCα. In addition, a decrease in Ach-induced phosphorylation of MYPT was observed in SMC from aged rat which possibly is due to age-related reduction in translocation of RhoA resulting in reduced activation of Rho-dependent kinase (ROCK). This leads to decreased phosphorylation of MLC_{20} leading to reduced contraction[54] as exhibited in the aged rats.[55] Furthermore, aged rat SMC showed decreased Ach-induced (i) phosphorylation of HSP27, (ii) association of actin with myosin as well as (iii) association of tropomyosin with HSP27.[56]

Chaperone proteins such as HSP27 are crucial to regulate protein conformation, and are essential to protect proteins from misfolding under normal conditions and when cells are exposed to stress.[57] The role of small heat shock proteins (sHSPs) in aging has been published by different groups.[58,59] For example over-expression of *Drosophila melanogaster* HSP22 (DmHsp22) in motor neurons resulted in a 30% increase in mean lifespan and an increase in resistance to heat and oxidative stresses. Interestingly, flies over expressing DmHsp22 kept their locomotors activity/fitness for longer and flies which do not express DmHsp22 were more sensitive to stress with decreased life span.[58] Most importantly, DmHsp22 has been also shown to have an effect on human fibroblast by extending their lifespan.[60] Also in the worm *Caenorhabditis elegans*, HSP16 has been shown to promote longevity by being co-activated by transcription factor (HSF1) and Daf-16.[61] Since several studies have shown a critical role of small heat shock proteins in aging, it will be important to analyze the mechanism and regulation of sHSPs during aging.

Restoration of smooth muscle contraction by reinstatement of signaling pathways

Ectopic expression of cav-1 as well as phosphorylated HSP27 in aged SMC reinstates smooth muscle contraction and force generation.[53] Ectopic expression of cav-1 mediates restoration of PKCα and RhoA signaling pathways while ectopic expression of phospho-HSP27 reinstates association of contractile proteins as well as PKCα -mediated signaling pathway.[54]

Aged transgenic mice that express phosphomimic HSP27 exhibited regular colonic motor function. Colon tissue from aged transgenic mice showed no changes in the expression levels of contractile associated proteins such as HSP27, HSP20, tropomyosin or caldesmon. However, there was a significant increase in Ach-induced phosphorylation of myosin light chain in CSMC from aged (24–32 months) transgenic mice. Colon tissue from aged transgenic mice expressing phosphomimic HSP27 showed reinstatement of adult-like contraction and relaxation pattern. The data suggests that reinstatement of phosphorylated HSP27 in CSMC from aged animals resulted in restoration of contraction and relaxation response similar to the adult.

Aging and Altered Endocrine/Paracrine regulation in smooth muscle cells

Growth factors are involved in proliferation and differentiation of smooth muscle cells originating from a variety of tissues, including the vasculature and the airways.62 Deletion of growth hormone (GH) receptors or GH (alone or along with other pituitary hormones) produces secondary suppression of circulating IGF-1 and insulin, robust extension of both median and maximal lifespan as well as numerous indications of delayed and/or slower aging. Direct targeting of insulin-like growth factor-1 (IGF-1) signaling can also extend longevity.63 Calorie restriction has been well accepted to delay aging and prolong life. CR decreases serum IGF-1 concentration by ~40%, protects against cancer and slows aging in rodents.

Several growth factors have been shown to induce a concentration-dependent contraction and to be potential inducers of contractile mediator release.64 The mechanism by which growth factors induce contraction has only been partly elucidated. IGF-1 is one such growth factor whose effect on smooth muscle contraction has been studied extensively. IGF-1 is a mechano-growth factor produced primarily by liver as an endocrine hormone as well as in target tissues in a paracrine/autocrine fashion. Recent evidence shows that IGF-1-receptor can activate the Rho/Rho kinase pathway directly65 and may be involved in vascular smooth muscle contraction via Rho-kinase.66 IGF-1 has been reported to induce a slowly developing sustained contraction, which was dependent on Rho-kinase, since contraction was almost completely inhibited by (+)-I-trans-4-(1-aminoethyl)-N-(4-pyridyl) cyclohexane carboxamide (Y-27632; 1 μM).67,68 It has also been demonstrated that IGF-1 increases intracellular calcium concentration $[Ca^{2+}]_I$ mediated by protein kinase C–dependent pathways, which modulate $[Ca^{2+}]_I$ through a Na^+/Ca^{2+}-dependent transporter. These intracellular signaling events have been suggested to be required for mitogenic and contractile actions of growth factors in vascular smooth muscle cells. Diabetic cardiomyopathy is characterized by impaired ventricular contraction and altered function of IGF-I. It has been shown that impaired cardiac excitation and contraction coupling in diabetes is significantly improved by exogenous IGF-I administration, which has been proposed to be probably by improved intracellular $[Ca^{2+}]_I$ homeostasis. IGF-1–induced cardiac contractile response was reduced with advanced age. It has been recently shown that hypertension and advanced age significantly attenuated IGF-1–induced myocardial force generating capacity indicating that IGF-1 may play a role in the altered myocardial function under hypertension and/or advanced age. The resistance to IGF-1 in hypertension and advanced age may be due to alterations in nitric oxide and $[Ca^{2+}]_i$ modulation.

Mechanisms Underlying Age-related Cellular Dysfunction: Insights from Extraintestinal Sites

Aging, Oxidative Stress and DNA Damage

Elucidating the molecular mechanisms that underlie the effects of physiological aging on organ and cellular function is an area of intense investigation. Perhaps the most frequently

proposed mechanism underlying cellular senescence is oxidative damage affecting the nucleotide pool and biochemical pathways associated with maintenance of cell structure and function.69 For example, the DNA damage observed in senescent cells has been attributed to elevated levels of reactive oxygen species (ROS) and failing DNA damage repair processes. The nucleotide pool appears to be a significant target for oxidants and oxidized nucleotides, once incorporated into genomic DNA, can lead to the induction of a DNA strand break-associated injury that triggers senescence in normal cells. However, it remains unclear how labile molecules such as ROS are able to damage chromatin-bound DNA to a sufficient extent to invoke persistent DNA damage and abnormal cell-cycle signaling.

Epigenomics and Aging

In addition to age-associated accumulation of damage to the nucleotide pool, emerging evidence supports a role for epigenetic mechanisms in cellular senescence.70 Epigenetics refers to a set of potentially self-perpetuating, covalent modifications of DNA and post-translational modifications of nuclear proteins that can produce lasting alterations in chromatin structure. These mechanisms include methylation of DNA that prevents gene transcription, acetylation of histone proteins that promotes gene transcription and a robust pool of endogenous microRNAs that regulate gene transcription. These mechanisms can result in alterations in specific patterns of gene expression that ultimately affect the ability of aged cells to be “plastic”. For example, genome-wide array analyses have implicated cell specific changes in histones, DNA methylation and regional relocation of RNAs as key processes underlying age-related changes in neuronal functions and synaptic plasticity.71 These experiments have revealed evolutionarily conserved gene clusters associated with aging including apoptosis-, telomere- and redox-dependent processes, insulin and estrogen signaling and water channels.

Signal transduction Pathways Implicated in Regulating Lifespan

Protein synthesis and degradation are regulated by distinct genetic pathways that control aging in numerous eukaryotic species. These highly conserved mechanisms include the insulin/IGF-1 (Insulin-like Growth Factor-1), TGF-I (Transforming Growth Factor-beta), JNK (c-Jun terminal kinase), RTK/Ras/MAPK (Receptor Tyrosine Kinase/ Ras/Mitogen-Activated Protein Kinase) and TOR (kinase Target of Rapamycin) signaling cascades and the mitochondrial respiratory system that promote protein synthesis, and the intracellular protein degradation machineries, including the ubiquitin-proteasome system and lysosome-mediated autophagy.72 Emerging evidence supports the existence of a complex intimate regulatory relationship between mechanisms promoting protein synthesis and those mediating protein degradation. Specifically, conditions that favor protein synthesis can enhance the rate at which damaged proteins accumulate. This may explain why targeted genetic interventions and environmental factors such as dietary restriction that reduce protein synthesis can extend lifespan and increase resistance to cellular stress in several experimental models of aging.

Aging and Autophagy

Autophagy or cellular self-eating is a major pathway for bulk degradation and recycling of damaged cytosolic macromolecules and organelles including mitochondria. Mitochondria play a pivotal role in energy production via the oxidative phosphorylation pathway and cell death via both extrinsic (cell-surface receptor mediated) and intrinsic apoptotic pathways. Reactive oxygen species (ROS) form as by-products of oxidative phosphorylation and their accumulation can cause mitochondrial damage. Dysfunctional mitochondria accumulate in aged individuals and cell homeostasis is maintained by removal of the damaged organelles by autophagy.73 If this process is insufficient cell death can occur via a process that may be distinct from classic apoptosis.74

Genetic studies indicate that autophagy-related genes (Atg) are required for lifespan extension in several eukaryotic models (including yeast, nematodes, flies and mammals) exposed to prolonged nutrient reduction. Promoting basal expression of Atg8 in Drosophila in the nervous system extends lifespan by 50%.75 These observations support the emerging view that autophagy may be a pivotal regulatory mechanism for aging in eukaryotic species.76

Recent evidence suggests that SIRT1, a mammalian homolog of Sir2 longevity factor, plays an important role in the regulation of metabolism, cellular survival, and lifespan. Among its functions SIRT1 appears to regulate the formation of autophagic vacuoles, either directly or indirectly through a downstream signaling network. The interactions of SIRT1 with the forkhead transcription factor (FoxO) and p53 signaling can also regulate both the autophagic degradation and lifespan extension supporting a potential pivotal role for autophagy in the regulation of lifespan.77

Another line of evidence supporting a key role for autophagy in regulating longevity is data that inhibition of the mammalian target of rapamycin (mTOR) significantly increases lifespan in several organisms and observations that long-term treatment with rapamycin, an inhibitor of the mTOR pathway78, or ablation of the mTOR target p70S6K79 extends lifespan in mice and may prevent congnitive deficits in a mouse model of Alzheimer's disease.80

The emerging role for cellular redox status and aging

Cellular processes such as proliferation, differentiation and death are intrinsically dependent upon the redox status of a cell. Among markers of redox flux, cellular NAD(H) levels play an important role in transcriptional reprogramming. The interdependence of changes in gene expression and NAD(H) is highly conserved and viewed as crucial for the survival of a species because of its role in determining reproductive capacity and longevity.81 Proteins that bind and/or use NAD(H) as a co-substrate (such as, CtBP and PARPs/Sirtuins respectively) are known to induce changes in chromatin structure and transcriptional profiles. The ability of these proteins to sense changes in NAD(H) levels has been implicated in their roles in development, stress responses, metabolic homeostasis, reproduction and aging. It appears that the levels and activities of these proteins, and the availability of NAD(H) are equally important.

Interventions that May Reduce the Effects of Aging

A substantial body of evidence covering yeast to humans indicates that dietary restriction is associated with longer life expectancy compared to organisms fed a normal or caloric rich diet.82 A similar effect is observed when the activity of nutrient-sensing pathways is reduced by mutations or chemical inhibitors. For example, in rodents, both dietary restriction and decreased nutrient-sensing pathway activity can lower the incidence of age-related loss of function and disease, including tumors and neurodegeneration. Dietary restriction also increases life span and protects against diabetes, cancer, and cardiovascular disease in rhesus monkeys, and in humans it is associated with changes that protect against these age-related changes. It is noteworthy that tumors and diabetes are also uncommon in humans with mutations in the growth hormone receptor, and natural genetic variants in nutrient-sensing pathways are associated with increased human life span.

The composition of the diet is likely important. For example, a recent study examined the effect of a hypoproteic (8% protein vs. 22% protein in controls) diet on myenteric neurons in the Wistar rat.83 Morphometric analysis of the nitrergic (NADH-diaphorase) positive myenteric neurons demonstrated a larger number of small neurons and a smaller number of

medium neurons in the animals receiving the hypoproteic chow, suggesting that the neuronal growth was affected by the diet.

The specific nutrient-sensing pathways involved in age-related diminution of function is an area of intense investigation. As mentioned previously, target of rapamycin (TOR) is an evolutionarily conserved nutrient-sensing ser/thr protein kinase network that regulates growth, metabolism and aging in eukaryotic cells.84،85 TOR is also emerging as a robust mediator of the protective effects of various forms of dietary restriction, which can extend life span and slow the onset of certain age-related diseases across species. TOR signaling may slow aging through downstream processes including mRNA translation, autophagy, endoplasmic reticulum (ER) stress signaling, stress responses, and metabolism.

Specific nutrients may have a protective effect on age-related diminution in neural function via their direct or indirect trophic actions. For example, polyunsaturated fatty acids (PUFA) of the omega-3 series and omega-6 series stimulate neurite outgrowth in immature DRG neurons obtained from male or female rats. Recently, the effects of omega-3 PUFAs, particularly docosahexanoic acid were shown to have a marked effect on neurite outgrowth in DRGs from aged (18–20 months) rats. These effects were comparable to that of nerve growth factor and all-trans-retinoic acid.86

Sialylated glycosphingolipids (i.e., gangliosides) and glycoproteins play important roles in cell contact, communication, and signaling processes and decreased sialylation is observed in advanced age and correlates with neurodegeneration.87 Feeding aged rats a sialic acid rich diet stimulated salivation and subpopulations of colon enteric neurons.88 Specifically, the colonic expression of nNOS and panneuronal marker UchL1 (PGP9.5) and the rate-limiting enzyme for sialic acid synthesis GNE ((glucosamine (UDP-*N*-acetyl)-2-epimerase/*N*-acetylmannosamine kinase; E.C. 5.1.3.14)) were differentially affected in young and aged rats by sialic acid feeding. The UchL1 gene expression showed a marked increase in aged control rats as compared to young control rats which normalized in aged rats to the level of young control rats. The nitrergic marker nNOS showed a trend to increased expression in old rats and sialic acid feeding strongly reduced nNOS expression. In contrast, the expression of the cholinergic neuron markers ACHE (acetylcholine esterase), calbindin 1, and calbindin 2 were neither affected by age nor sialic acid feeding.

A novel potential target for the development of anti-aging drugs is the SIR2 (silent information regulator 2) family of NAD-dependent deacetylases/ADP-ribosyltransferases, called "sirtuins." Sirtuins regulate many fundamental biological processes in response to a variety of environmental and nutritional stimuli. The mammalian SIR2 ortholog SIRT1 has received attention recently, and small molecule SIRT1 activators (STACs), including the plant-derived polyphenolic compound resveratrol, have been developed.89 Sirtuin activity is also regulated by NAD biosynthetic pathways, and nicotinamide phosphoribosyltransferase (NAMPT) plays a critical role in the regulation of mammalian sirtuin activity. Recent studies have provided a proof of concept that nicotinamide mononucleotide (NMN), the NAMPT reaction product, can be used as a nutriceutical to activate SIRT1 activity and, thereby, modulate longevity pathways. 89

Summary

Understanding the effects of aging on the gut neuromuscular axis is of growing and profound importance in light of demographic data demonstrating a steady increase in the aged population globally. Likely areas of significant importance include the potential effects of aging on the extrinsic and intrinsic innervations, smooth muscle function in the human colon, and determination of whether specific biomarkers can be identified in the gut neural plexi, ICC, glial cells or smooth muscle that predict the onset of neurodegeneration. Age-

related neuronal losses occur in both the myenteric plexus and submucosal plexus. In general, these losses start in adulthood and progress during the lifespan and involve cholinergic neurons predominately. Parallel age-dependent loss of enteric glia is also observed. The drop-out of these cell populations follows an oral-anal gradient, with greater losses noted distally. Dystrophic axonal swelling and dilated varicosities also increase in an age-dependent manner in the extrinsic innervations (vagal, DRG and sympathetic pathways), as well as, nitrergic neurons in the gut.

Advanced age is associated with increased oxidative stress and mitochondrial dysfunction at the cellular level, culminating in disequilibrium between cytoprotective and cytotoxic pathways favoring the latter. Caloric restriction and/or diets supplemented with specific nutrients may help attenuate the effects of aging on neuromuscular function.

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