Aging and Neural Control of the GI Tract
V. Aging and gastrointestinal smooth muscle: from signal transduction to contractile proteins

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Bitar, Khalil N. Aging and Neural Control of the GI Tract. V. Aging and gastrointestinal smooth muscle: from signal transduction to contractile proteins. Am J Physiol Gastrointest Liver Physiol 284: G1–G7, 2003; 10.1152/ajpgi.00264.2002.—The object of this theme is to offer new perspectives on the effect of aging on signal-transduction pathways associated with agonist-induced contraction of smooth muscle cells from the colon. Smooth muscle cells from old rats (32 mo old) exhibit limited cell length distribution and diminished contractility. The observed reduced contractile response may be due to the effect of aging on signal-transduction pathways, especially an inhibition of the tyrosine kinase-Src kinase pathway, a reduced activation of the PKC pathway, and a reduced association of contractile proteins [heat shock protein 27 (HSP27)-tropomyosin, HSP27-actin, actin-myosin]. Levels of HSP27 phosphorylation are also reduced compared with adult rats.

AGING AFFECTS SMOOTH MUSCLE contraction in different tissues. A tremendous amount of work has been cited in the literature on the effect of aging on skeletal muscle or on the cardiovascular system, yet very little work has been done on gastrointestinal smooth muscle. There is considerable evidence that the incidence of certain problems, such as dysphagia and constipation, increases dramatically with age. The areas at greatest risk of developing aging-related dysfunction are the upper gastrointestinal (GI) tract, particularly the oropharynx and esophagus, and the distal tract (colon and rectum). The amplitude and duration of the peristaltic pressure wave in hypopharyngeal sphincter of elderly humans are increased, but the amplitude of the upper esophageal sphincter is decreased (18). A decrease in maximal force and lower maximal velocity of shortening is observed in longitudinal smooth muscle from urinary bladder of aging rats (12). In colonic smooth muscle cells from aging rats, a decrease in calcium and potassium channel currents affects the initiation of contraction (25). Significant effects of aging on gastric and colonic motility in rats include slow gastric emptying of liquids and decreased fecal pellet transit and production (19). The age-dependent changes are both in the cholinergic neurotransmission as well as the response of smooth muscle to acetylcholine.

In this theme review we have focused on the effect of aging on signal-transduction pathways and on the association of contractile proteins in rat colon smooth muscle cells. In smooth muscle cells, receptor activation of PKC results in activation of sphingomyelinase and a concomitant production of ceramide (6, 17). Ceramide induces a sustained contraction of smooth muscle cells through a pathway involving the activation of MAP kinase and also through the activation of a non-receptor tyrosine kinase pathway [activation of pp60Src and phosphatidylinositol 3-kinase (PI3)-kinase] (21).

CHANGES OF FIBER TYPE COMPOSITION DURING AGING

Most of the studies relating to this topic are from the cardiovascular system. Cardiovascular function is altered with age in men and women. Advancing age is a risk factor for the development of cardiovascular disease. With advancing age, vascular function and reactivity are impaired, resulting in increased stiffness, decreased distensibility and compliance, and wall thickening (10).

Smooth muscle cells from the colon of old rats (32 mo old) exhibit limited cell-length distribution and diminished contractility. Figure 1A shows the percent decrease in cell length in response to acetylcholine or ceramide (0.1 μM) in adult smooth muscle cells (35.7 ± 2.9%, n = 3; P < 0.01) compared with aging smooth muscle cells (16.3 ± 2.6%, n = 3; P < 0.05). Figure 1B displays the length distribution of smooth muscle cells from the colons of rats. The distribution profile of cells from adult rats distributed over a wider range than cells from aging rats. In the unstimulated state, the
average cell length in aging rats (45.3 ± 5.4 μm, n = 150; P < 0.05) is shorter (~25%) than cells from adult rats (59.5 ± 6.0 μm, n = 150; P < 0.05). Smooth muscle cells from adult rat (12 mo old) had a span of cell-length distribution (37–70 μm) with 55% in the range of 40–60 μm and 44% longer than 60 μm. Aging rat smooth muscle cell length varies from 26 to 57 μm, with 68% longer than 40 μm. Average cell length in the adult and aging rats is 59.5 ± 6.0 (n = 150; P < 0.05) and 45.3 ± 5.4 μm (n = 150; P < 0.05), respectively. C: distribution of muscle cell lengths in the control state and after 4-min exposure to ceramide (0.1 μM). Shift of curves to the left indicates disappearance of relaxed (elongated) cells and appearance of contracted (short) cells. Muscle cells in adult rats exhibited a bigger shift of the curve to the left (Fig. 1B) than the response to ceramide in old rats (Fig. 1C).

TYROSINE KINASE PATHWAY

Signal-transduction pathways mediating contraction of smooth muscle cells involve cascades of protein phosphorylation. The changes of protein kinases in aging cells leading to aberrant protein phosphorylation have been reported in other cell systems. An increased activation of EGF-receptor tyrosine kinase by EGF and
transforming growth factor-α in the colonic mucosa of aged rats has been described. Increased expression of pp60cSrc was reported in gastric mucosa of aged rats (9).

Ceramide activates a tyrosine kinase-Src kinase-PI3-kinase pathway in smooth muscle cells from the rabbit colon (6), and PI3-kinase seems to be a preferential substrate for pp60Src kinase. Figure 2 shows that ceramide was activated in response to ceramide in colon smooth muscle cells of adult and aging rats (16.5 ± 2.2% increase, n = 3; P < 0.001) but not in aging rats (4.0 ± 0.6% increase, n = 3; P = 0.12). P85 subunit is present in both type IA and type IB PI3-kinase, but the amounts and ratio of these two types are unknown. The catalytic subunits of PI3-kinase (P110α and P110γ) are activated in aged rats. Ceramide has been shown to activate type IA (P110α) PI3-kinase but not type IB (P110γ) PI3-kinase in rabbit smooth muscle cells (21). Figure 2 shows that P110α of PI3-kinase was activated in response to ceramide in aging rats (16.5 ± 2.2% increase, n = 3; P < 0.05) but to a much lesser extent than in adult rats (41.5 ± 5.3% increase, n = 3; P < 0.005). Similar data were observed for P110γ subunit of PI3-kinase: adult rats (63.9 ± 4.7% increase, n = 3; P < 0.005) in adult rats vs. (30.1 ± 5.1% increase, n = 3; P < 0.05) in aging rats. The data suggest that the "tyrosine kinase-Src kinase" pathway was inhibited in smooth muscle cells of aging rats.

In colonic smooth muscle cells, ceramide-induced contraction as well as ceramide-induced activation of Src kinase (6) is Ca²⁺ dependent. Intracellular Ca²⁺ levels play an important role in modulating contraction-relaxation coupling of muscle cells. Age-associated decrease in intracellular calcium level in aging colon smooth muscle cells has been previously reported. Several studies suggest that aging affects either the number and/or functional properties of calcium channels in neurons of rat brain (11) and in muscle cells of rat heart (15). Inhibition of Src-kinase in response to ceramide in aging colon smooth muscle cells is probably due to a lower level of intracellular Ca²⁺ resulting from either/both the defect of extracellular calcium influx and/or a defect in calcium release from intracellular calcium stores. This suggestion needs to be confirmed in GI smooth muscle.

EFFECT OF AGING ON K CHANNELS

Another topic has seldom been investigated in GI smooth muscle, compared with the abundance of work in cardiovascular and corporal smooth muscle. In general, modifications in expression of ion channels that regulate cell excitability most likely contribute to an impaired cell function in older people.

One characteristic of aging coronary arteries is their enhanced contractile response to endothelial vasoconstriction factors, which increase the risk of coronary vasospasm in older people. There is evidence that aging induces increased responses of rat coronary and mesenteric arteries to K⁺ (13). The increase in K⁺-induced contractions in aging animals suggests a change in K⁺-channel function or expression as age.

**Fig. 2.** Phosphorylation of Src kinase and phosphatidylinositol 3 (PI3)-kinase subunits (P85 regulatory subunit and P110α and P110γ catalytic subunits) in response to ceramide in colon smooth muscle cells of adult and aging rats. A: protein lysate (100 μg) of untreated cells or cells stimulated with ceramide (0.1 μM) were subjected to SDS-PAGE and Western blotted with phosphospecific monoclonal pp60Src kinase antibody. pp60Src Kinase was phosphorylated on agonist stimulation in adult rats but not in old rats. B-D: immunoprecipitates (IP) of phosphotyrosine antibody (IP: anti-p-tyr antibody) from 200 μg of protein lysate of untreated cells or cells stimulated with ceramide (0.1 μM) were subjected to SDS-PAGE and Western blotted with either P85 monoclonal antibody (IB: anti-P85 antibody; B) or P110α polyclonal antibody (IB: anti-P110α antibody; C) or P110γ polyclonal antibody (IB: anti-P110γ antibody; D). The P85 regulatory subunit of PI3-kinase was activated in adult rats but not in aging rats, whereas P110α and P110γ catalytic subunits of PI3-kinase were activated more in adult rats than in aging rats.
progresses. Coronary arteries possess several types of K+ conductances; the large-conductance, voltage-dependent, and Ca2+-activated K+ channel (MaxiK) is particularly abundant and plays a key role in regulating arterial tone. (22).

MaxiK channels are diminished in aging coronary arteries in rats and humans. A diminution in the numbers of MaxiK channels leads to a decrease in the normal tonic hyperpolarizing force provided by the activity of these channels in coronary arteries and thus may contribute to the increased risk of coronary spasm in older people. Under physiological conditions, MaxiK channels are tonically active, serving as a hyperpolarizing force that opposes contraction. Thus reduced expression of MaxiK channels in aged coronary arteries would lead to a decreased vasodilating capacity and increased risk of coronary spasm and myocardial ischemia in older people.

Similarly, in corporal smooth muscle, MaxiK channels also seem to diminish with age. The mechanism for this tissue-specific aging-related change in channel density is unknown (23). It would be interesting to determine whether sexual hormones that diminish during aging (7) trigger these changes and whether such changes are contributing factors in GI smooth muscle.

**EFFECT OF AGING ON REGULATION OF Ca2+ IN COLONIC SMOOTH MUSCLE CELLS**

The major mechanisms involved in smooth muscle contractions not associated with changes in membrane potential are the release of inositol 1,4,5-trisphosphate and the regulation of Ca2+ sensitivity. Both mechanisms are important for the initial and sustained contractile response in colonic smooth muscle.

As in striated muscle, the amount of intracellular free Ca2+ is the key to regulation of smooth muscle tone. In the smooth muscle cells, Ca2+ binds to calmodulin, which is in contrast to striated muscles, in which intracellular Ca2+ binds to the thin filament-associated protein troponin (3). The calcium-calmodulin complex activates myosin light chain kinase (MLCK) by association with the catalytic subunit of the enzyme. The active MLCK catalyzes the phosphorylation of the regulatory light chain subunits of myosin (MLC20). Phosphorylated MLC20 activates myosin ATPase, thus triggering cycling of the myosin heads (cross-bridges) along the actin filaments, resulting in contraction of the smooth muscle. A decrease in the intracellular level of Ca2+ induces a dissociation of the calcium-calmodulin MLCK complex, resulting in dephosphorylation of the MLC20 by MLC phosphatase and in relaxation of the smooth muscle.

An age-related impairment of intrinsic sarcoplasmic reticulum (SR) function, i.e., the rate of Ca2+ uptake and the fractional rate of SR filling, and a decrease in SR volume are the most probable factors underlying the decreased speed of contraction in old fast-twitch motor units (8). Uncoupling of sarcoplasmic excitation and SR Ca2+ release is assumed as a major determinant of weakness and fatigue. With increasing age, an increase of the number of RYR1 ryanodine receptor uncoupled from dihydropyridine receptor has been found in rat (soleus and extensor digitorum longus muscle) and human (vastus lateralis muscle). Dihydropyridine receptor RYR1 uncoupling leads to a significant reduction in the amount of releasable Ca2+ in skeletal muscles from old animals and humans. Similar studies would yield valuable information in GI smooth muscle.

In smooth muscle, the force/Ca2+ ratio is variable and depends partly on specific activation mechanisms. The effect of calcium-sensitizing agonists are mediated by GTP-binding proteins that generate protein kinase C or arachidonic acid as second messengers. The major mechanism of Ca2+ sensitization of smooth muscle contraction is through inhibition of the smooth muscle myosin phosphatase, thus increasing MLC20 phosphorylation by basal level activity of MLCK. The resulting myosin phosphorylation and subsequent smooth muscle contraction therefore occurs without a change in sarcoplasmic Ca2+ concentration. Ca2+ sensitization by the RhoA/Rho kinase pathway contributes to the sustained phase of the agonist-induced contraction in smooth muscle.

![Fig. 3. Differential ceramide-induced activation of PKC subunits (α, β, and γ) in smooth muscle cells from adult and aging rats. Protein lysates of particulate fraction of untreated cells or cells stimulated with ceramide (0.1 µM) were subjected to SDS-PAGE and Western blotted with phosphospecific monoclonal PKC-subunit antibodies. PKCα, β, and γ were activated in response to ceramide in adult rats, whereas only PKCγ was activated in response to ceramide in aging rats.](http://ajpgi.physiology.org/)

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Rho kinase is known to inhibit MLC phosphatase and to directly phosphorylate MLC, altogether resulting in a net increase in activated myosin and the promotion of muscle contraction. Rho kinase antagonist Y-27632 would be a good tool to investigate the effect of aging on the response of colonic smooth muscle to contractile agonists.

**EFFECT OF AGING ON AGONIST-INDUCED ACTIVATION OF PKC**

PKC has been shown to be activated in different cell systems such as in colon smooth muscle cells (5), vascular smooth muscle cells, atrial myocytes, neonatal rat hearts. On stimulation with contractile agonists, PKC-α translocates to the cell membrane in adult rabbit colon smooth muscle cells (5) and associates with translocated RhoA and heat shock protein (HSP27) (1, 2). In old rats, (Fig. 3) the γ-isofrom of PKC is activated in aging rats, whereas three isoforms of PKC (α, β, and γ) are activated in normal adult rats.

A class of proteins termed receptors for activated C kinases (RACKs) has been described that bind PKC. A functional impairment in PKC by RACK1 deficiency in aged rat brain cortex has been reported (14). There is a possibility that, in aging rats, the inhibition of PKC activation may be affected by the dysfunction of RACKs.

**EFFECT OF AGING ON “PKC-MAP” KINASE PATHWAY**

We have previously shown that in smooth muscle cells, contractile agonists, activate MAP kinase (17), and ceramide-induced MAP kinase activation is parallel, yet downstream, of Src and PI3-kinase activation (6). Western immunoblotting, using a phosphospecific MAP kinase antibody, indicates that MAP kinase is phosphorylated to a lesser extent in aging rats (16.5 ± 1.9% increase, n = 3; P < 0.005) than in adult rats (38.1 ± 0.5% increase, n = 3; P < 0.005). The difference in the activation of the PKC isoform may result in less activation of MAP kinase in aged rats, which would be also due to the lack of activation of the “tyrosine-Src kinase” pathway.

![Graph showing percentage increase in HSP27 phosphorylation](image_url)
EFFECT OF AGING ON THE ASSOCIATION OF THE CONTRACTILE PROTEINS (HSP27-TROPOMYOSIN, HSP27-ACTIN, ACTIN-MYOSIN) AND ON HSP27 PHOSPHORYLATION

Changes in contractile response of smooth muscle could also be due to changes in the amount of contractile proteins and their association with one another. In the intimal cells of human coronary arteries, the amount of desmin decreases from childhood to adulthood and disappears in the elderly. In aortic smooth muscle cells of aging rats, the percentages of myosin and desmin decreases compared with young rats, whereas actin and vimentin remained unchanged, as well as an age-related increase in the proportion of the α-actin isoform. We have identified a low-molecular weight heat shock protein, HSP27, which may be a putative contractile protein mediating a PKC-dependent contraction in rabbit colonic smooth muscle cells (5). An interaction between HSP27 and actin, as well as an interaction between HSP27 and contractile proteins such as myosin, tropomyosin, and caldesmon, was suggested in colonic smooth muscle cells. HSP27 plays a role in cytoskeletal reorganization by increasing its stability and forms a complex with other contractile proteins such as actin, myosin, caldesmon, and tropomyosin. Our recent study (1) suggests an interaction among HSP27, tropomyosin, and actin. On contraction induced by acetylcholine or ceramide (0.1 μM), the amount of actin immunoprecipitated with HSP27 increased, suggesting a functional relationship between the association of contractile proteins and agonist-induced contraction.

The expression of heat shock proteins has been shown to be markedly reduced with age in both cultured cells and in vivo (4), suggesting a defective protective mechanism with aging. In intact animals, hsp70 gene expression induced by the stress of restraint is also reduced with aging in rat aorta, vena cava, and adrenal glands when 6- and 24-mo-old Fischer rats were compared (24).

Immunoprecipitation followed by Western immunoblotting was applied to detect the associations among contractile proteins. Figure 4 shows that the amount of tropomyosin immunoprecipitated with HSP27 antibody increased on agonist stimulation in both groups of rats but much more in adult rats (32.1 ± 1.1% increase, n = 3; P < 0.005) than in aged rats (9.3 ± 1.6% increase, n = 3; P < 0.05). A similar result was obtained with immunoprecipitation of actin with HSP27. The amount of actin immunoprecipitated with HSP27 antibody increased on agonist stimulation in both groups of rats but much more in adult rats (31.1 ± 2.9% increase, n = 3; P < 0.005) than in aged rats (12.9 ± 0.8% increase, n = 3; P < 0.05). The amount of myosin immunoprecipitated with actin antibody increased on stimulation in both groups of rats but much more in adult rats (51.3 ± 1.2% increase, n = 3; P < 0.005) than in aged rats (27.9 ± 3.3% increase, n = 3; P < 0.05). The ∼50% decrease in actin-myosin association in aged and adult rats correlates with the percent decrease in shortening, as shown in Fig. 1A. Ceramide and acetylcholine induce phosphorylation of HSP27 in colonic smooth muscle cells (1). Ceramide-induced phosphorylation of HSP27 was reduced more in old rats (15.3 ± 6.2% increase, n = 3; P < 0.05) than in adult rat smooth muscle cells (14.2 ± 13.6% increase, n = 3; P < 0.001; Fig. 5). This may be due to an inhibitory effect of aging on the tyrosine kinase-Src kinase pathway and on the “PKC pathway.”

SUMMARY

Changes in the upstream cascade leading to the association of contractile proteins would affect contractility. Scarce data are available as to the effect of aging on GI smooth muscle. We have proposed a model whereby the association of HSP27 with other contractile proteins is an integral mechanism of PKC-mediated smooth muscle contraction. Inhibition of MAP kinase activation in aged rats may result in a decrease in HSP27 phosphorylation. MAP kinase phosphorylates MAPKAPK2 kinase, which further phosphorylates heat shock protein (HSP27) (16, 20). Reduced phosphorylation of HSP27 affects the associations of contractile proteins, leading to less contractility in aged smooth muscle.

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REFERENCES


