HIGHLIGHTED TOPIC | Mechanisms and Modulators of Temperature Regulation

Peripheral mechanisms of thermoregulatory control of skin blood flow in aged humans

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Holowatz LA, Kenney WL. Peripheral mechanisms of thermoregulatory control of skin blood flow in aged humans. J Appl Physiol 109: 1538–1544, 2010. First published April 22, 2010; doi:10.1152/japplphysiol.00338.2010.—Human skin blood flow is controlled via dual innervation from the sympathetic nervous system. Reflex cutaneous vasoconstriction and vasodilation are both impaired with primary aging, rendering the aged more vulnerable to hypothermia and cardiovascular complications from heat-related illness. Age-related alterations in the thermoregulatory control of skin blood flow occur at multiple points along the efferent arm of the reflex, including 1) diminished sympathetic outflow, 2) altered presynaptic neurotransmitter synthesis, 3) reduced vascular responsiveness, and 4) impairments in downstream (endothelial and vascular smooth muscle) second-messenger signaling. This mechanistic review highlights some of the recent findings in the area of aging and the thermoregulatory control of skin blood flow.

AGING AND REFLEX VASOCONSTRICTION

Reflex cutaneous vasoconstriction is an early and sustained response to whole body cold exposure, limiting convective heat loss to the environment. This thermoregulatory response is markedly impaired in aged skin (8, 11, 20, 22, 71). Attenuated reflex vasoconstriction contributes to an inability to maintain body core temperature (Tc) during exposure to even mild cold stress (22°C) in aged humans (11). Compromised vasoconstriction in aged skin is due to impaired mechanisms at multiple points along the efferent arm of the sympathetic reflex, including 1) depressed autonomic skin sympathetic nerve activity (SSNA); 2) alterations in axonal synthesis and packaging of norepinephrine (NE) and putative cotransmitters, including neuropeptide Y and adenosine triphosphate (ATP); 3) reduced end-organ vascular responsiveness to these adrenergic neurotransmitters; and 4) alterations in downstream second-messenger signaling in the cutaneous vascular smooth muscle (Fig. 2).

Sympathetic Autonomic Dysfunction

Sympathetic efferent outflow in response to a cold stimulus is decreased with aging. When integrated SSNA was recorded in young (18–29 yr), middle-aged (38–51 yr), and elderly subjects (65–81 yr), the cold-induced increase in vasomotor nerve activity (bursts/min) was depressed by ~60% in the elderly group compared with the other two groups (22). Thus the sympathetic efferent signal for cold-induced cutaneous vasoconstriction is significantly weaker in aged skin.

Impaired Neurotransmitter Synthesis/Packaging/Release

In addition to the attenuated sympathetic stimulation of perivascular nerves and subsequent release of sympathetic transmitters, the relative contributions of these transmitters is also impaired with aging (Fig. 2). Reflex constriction in young adults, rendering older men and women more susceptible to cold- and heat-related illness. Age-related alterations in the thermoregulatory control of skin blood flow occur at multiple points along the efferent arm of the reflex, including 1) diminished sympathetic outflow, 2) altered presynaptic neurotransmitter synthesis, 3) reduced vascular responsiveness, and 4) impairments in downstream (endothelial and vascular smooth muscle) second-messenger signaling. This mechanistic review highlights some of the recent findings in the area of aging and the thermoregulatory control of skin blood flow.

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skin is mediated by both NE (60%) and cotransmitters (40%) (65, 66); however, the cotransmitter portion of constriction is abolished in aged skin (71). Thus older subjects rely entirely on impaired NE pathways to mediate the reflex vascular response to cold (71). The decrements in NE- and cotransmitter-mediated constriction are due, in part, to age-related decreases in transmitter synthesis and/or release (9, 15, 20).

One recently explored mechanism underlying age-related impairments in prejunctional neurotransmitter synthesis is the bioavailability of tetrahydrobipterin (BH4). BH4 is critical for optimal reflex vasoconstrictor function because it is required for the hydroxylation of tyrosine to L-3,4-dihydroxyphenylalanine by tyrosine hydroxylase (TH) in the prejunctional biosynthesis of catecholamines, as well as a powerful reductant that maintains TH in a reduced, active form (Fig. 2) (21, 59). However, both de novo synthesis and recycling pathways for BH4 are particularly vulnerable to oxidation, and, therefore, BH4 bioavailability decreases with increased oxidant stress. Because human aging is associated with a systemic net increase in reactive oxygen species (ROS) and reactive nitrogen species due to both increased production and decreased clearance (12, 44), a decrease in BH4 bioavailability may result. When BH4 is locally delivered to the cutaneous vasculature (by intradermal microdialysis), both physiologically (whole body cooling) and pharmacologically induced (tyramine) vasoconstriction are enhanced in aged skin (46). Furthermore, enhancing substrate availability by localized supplementation with tyrosine also augmented cutaneous vasoconstriction, with no additive effect of combining BH4 and tyrosine (47). These data suggest that optimal TH function is required to fully express the cutaneous vasoconstrictor response, and that reduced functional substrate and cofactor for TH contributes to the attenuated vasoconstriction in aged skin.

**Blunted α-Adrenergic Receptor Responsiveness**

In addition to a muted sympathetic signal and reduced NE (and functionally absent cotransmitter) synthesis and release, the end-organ vasoconstrictor response is also attenuated in aged skin (20, 43, 45, 69–71). NE-mediated constriction is significantly impaired in aged skin, with elderly subjects exhibiting a blunted constrictor response to both physiological and maximal doses of NE (70, 71). Evidence from other vascular aging models suggests that neuropeptide Y-mediated vasoconstriction undergoes a similar reduction (45), and ATP-mediated constriction is profoundly depressed (43). To date, the in vivo human data indicate that aged skin relies entirely on attenuated NE-mediated vasoconstriction to induce reflex vasoconstriction.

**Second-messenger Signaling**

The final potential site of age-related alteration in reflex vasoconstriction is the second-messenger signaling pathways that couple adrenoreceptor activation with the vascular smooth muscle contraction. Recently, the relative contribution of Rho kinase (ROCK)-dependent pathways in reflex cutaneous vasoconstriction has been explored. ROCK can be stimulated by NE and/or, in the case of localized cooling of the skin, by mitochondrial generation of superoxide (Fig. 2) (2, 3). Once activated, ROCK elicits vasoconstriction through two distinct mechanisms, including 1) inhibition of myosin light chain phosphatase, thereby maintaining myosin light chain phosphorylation without additional calcium (Ca2+) influx (Ca2+ sensitization); and 2) inducing the translocation of α2c-receptors from the Golgi apparatus to the cell membrane. In aged human skin, inhibition of ROCK significantly attenuated the reflex vasoconstrictor response and vasoconstriction induced by exogenous administration of NE (48). These data suggest that older skin relies on ROCK-dependent pathways (~50% of the total vasoconstrictor response) to a greater extent than their younger counterparts. Interestingly, this alteration in second-messenger signaling may be due to globalized pathological changes in microvascular function induced by aged-related increases in oxidant stress.

Cumulatively, these findings suggest that the age-associated decrement in thermoregulatory reflex vasoconstriction is attributable to several factors: 1) reduced efferent sympathetic signal, 2) reduced sympathetic synthesis and release of NE through decreased BH4 and tyrosine bioavailability, 3) a functional loss of sympathetic cotransmitters function, 4) a significant loss of end-organ sensitivity to NE, and 5) alterations in second-messenger signaling, including a greater dependence on ROCK-mediated pathways (see Fig. 2).
AGING AND REFLEX VASODILATION

Human aging in the absence of overt pathology is also associated with attenuated cutaneous vasodilation during thermal stress (37). The age-related reduction in SkBF during hyperthermia appears to be of peripheral origin due to decreased perivascular nerve stimulation. A decrease in axonal tetrahydrobiopterin (Bh4) and tyrosine bioavailability contributes to the documented decrease in neuronal synthesis and release of norepinephrine (NE). Reduced synthesis and/or release of neuropeptide Y (NPY) and potentially adenosine triphosphate (ATP) may contribute to the documented complete loss of sympathetic cotransmitter function. The end-organ vasoconstrictor response mediated by postsynaptic α-adrenergic, Y1, and P2X receptors (for NE, NPY, and ATP, respectively) is reduced, although it is unclear whether this loss of sensitivity is due to changes in receptor population, intracellular second-messenger signaling pathways, or a combination of the two. Finally, there are alterations in vascular smooth muscle second-messenger signaling, including an increased reliance on Rho-kinase-dependent mechanisms in aged skin. See text for details. H2O2, hydrogen peroxide; ONOO⁻, peroxynitrite; O₂⁻, superoxide; BH2, dihydrobiopterin; DA, dopamine; TH, tyrosine hydroxylase; αARs, adrenoceptors; Y1, NPY receptor; P2X, puridine receptor; DAG, diacylglycerol; IP3, inositol triphosphate; CaM, calmodulin; ROCK, Rho-kinase; P, phosphate; GTP, guanosine triphosphate; GDP, guanosine diphosphate; VC, vasoconstriction; VD, vasodilation; L-DOPA, L-3,4-dihydroxyphenylalanine; GEF, guanine nucleotide exchange factor; GAPs, GTPase-activating proteins; MLC, myosin light chain.

Sympathetic Dysfunction

Efferent sympathetic outflow in response to a mild heat stress stimulus is likely reduced with aging, but this decrement is not as well defined or characterized as that associated with cold stress. Grassi et al. (22) recorded SSNA during mild heat stress and found that the aged (65–81 yr) had an attenuated reduction in the integrated neurogram signal (47). Most data are difficult to interpret, because single-unit nerve signals were normally nitric oxide (NO)- and cyclooxygenase (COX)-mediated pathways. Both of these pathways have been important molecular targets from a vascular aging and health perspective and have significant potential for the development of successful intervention strategies. Finally, recent evidence suggests a novel role for platelet activation in reflex vasodilation that is significantly inhibited by commonly prescribed anti-platelet agents (26).
not obtained. The integrated multiunit neurogram is a combination of vasoconstrictor, vasodilator, piloerector, and sudomotor fibers. Furthermore, the subjects were only mildly heated during this study. Nevertheless, the aged subjects did display an altered SSNA response to heating, suggesting some degree of sympathetic vasodilator dysfunction.

**Acetylcholine**

The functional contribution of cholinergic cotransmitter(s) to reflex vasodilation is absent in aged skin until \( T_c \) is significantly elevated (\( \Delta T_c \geq 0.9°C \)) (24). Acetylcholine released from sympathetic cholinergic nerves is capable of modulating the initial rise in SkBF in the early phase of active vasodilation (63), and aged subjects display reduced SkBF in this \( T_c \) range. Studies using exogenous administration of acetylcholine (at a concentration that induced an increase in SkBF similar in magnitude to that observed during the initial phase of reflex vasodilation) indicate that vascular sensitivity at this concentration is not decreased in aged skin, but the relative contributions of the downstream second-messenger systems are altered. Specifically, COX-dependent vasodilation is attenuated, and endothelium-derived hyperpolarization factor-dependent vasodilation is augmented (33), suggesting that impaired COX-dependent second-messenger signaling may contribute to reduced reflex vasodilation in aged cutaneous vasculature.

**COX-dependent Mechanisms**

In young healthy humans, COX-derived vasodilators contribute to reflex vasodilation independent of NO (51). In contrast, with healthy aging, acute inhibition of COX does not alter the SkBF response during hyperthermia, suggesting that COX-derived vasodilators do not contribute to reflex vasodilation (25). There are several possible explanations for this finding, involving differential synthesis of COX-derived vasoactive products and vascular smooth muscle receptor activation with aging. First, COX is capable of synthesizing several active products and vascular smooth muscle receptor activation induces vasoconstriction and inositol phosphatase (IP) receptor activation induces vasodilation (Fig. 3) (17). With aging, as well as with several documented vascular pathologies, the expression of these vascular smooth muscle receptors shifts to increased TP and reduced IP receptors, respectively (17, 34, 53, 68). Further research utilizing specific thromboxane synthase and TP and IP receptor antagonists are needed to fully elucidate these mechanisms.

**NO-dependent Mechanisms**

In young healthy humans, NO contributes significantly to the increase in SkBF during hyperthermia. Due to the reduction in cotransmitter function, the aged rely primarily on impaired NO-dependent mechanisms to increase SkBF during hyperthermia (24). Optimal NO synthase (NOS) function for NO production depends on adequate substrate (L-arginine) and cofactor (BH4) availability. The intracellular localization of NOS in relation to the L-arginine subdomains (substrate pools) is also important (72). One potential mechanism limiting L-arginine...
arginase for NO synthesis and full expression of reflex cutaneous vasodilation in the aged is through upregulation of vascular arginase. Arginase is the last enzyme of the urea cycle and catalyzes the conversion of l-arginine to l-ornithine and urea, precursors for polyamine and collagen synthesis and thus vascular smooth muscle remodeling (16, 58). In the vasculature, arginase preferentially utilizes the common NOS substrate, l-arginine, limiting substrate availability for NO synthesis through NOS (Fig. 3). Arginase is upregulated in several animal models of endothelial dysfunction, including aging, atherosclerosis, and hypertension (4, 13, 14, 61); however, the mechanisms underlying the arginase upregulation are pathology specific. In line with these animal models of vascular dysfunction, acute arginase inhibition or direct l-arginine supplementation to the cutaneous vasculature significantly augments reflex vasodilation in aged human skin (31). Interestingly, arginase inhibition or l-arginine supplementation (or combined) increased SkBF similarly (~45% maximum SkBF), suggesting that the l-arginine-NOS pathway was saturated (at maximal NOS activity), and/or the cutaneous vessels were maximally vasodilated for the given degree of hyperthermic stress. Collectively, these results suggest that upregulated arginase limits NO production, and increasing l-arginine availability for NO synthesis can augment reflex vasodilation in aged skin (4, 29, 62, 73).

Another potential mechanism for reduced NO bioavailability with human aging is an increase in oxidant stress contributing to an increase in NO breakdown. In aged human skin, maximal cutaneous vasodilation to an exogenous NO donor is attenuated, suggesting either a relative inability of vascular smooth muscle to respond to NO through cyclic GMP mechanisms, or that NO may be rapidly degraded by ROS (52), possibly forming pro-constrictor oxidants (3). Endogenous ROS increase with aging and are capable of readily reacting with NO to form peroxynitrite (ONOO−), which limits NO-dependent vasodilation and may potentially serve as a vasoconstrictor stimulus (19).

In humans, high-dose nonspecific antioxidant supplementation (ascorbate) is commonly used to examine effects of oxidant stress on NO-dependent vasodilation. The advantage of using ascorbate is that it is water soluble, can be given in high concentrations in humans, and does not affect NOS activity. In aged human skin, localized high-dose ascorbate supplementation modestly augmented vasodilation with moderate increases in body Tc (ΔTc > 0.7°C); however, when ascorbate supplementation was combined with acute arginase inhibition, there was a further increase in SkBF (32). In contrast, localized ascorbate and/or arginase inhibition do not alter reflex vasodilation in young subjects. These results suggest that increased oxidant stress and upregulated arginase activity both limit NO bioavailability in aged skin. Furthermore, these mechanisms may be functionally linked through the uncoupling of NOS. Under circumstances where l-arginine and/or essential cofactors BH4 are limited, NOS can uncouple to produce superoxide instead of NO (19). In this positive feedback loop, NO reacts with superoxide forming ONOO−, which then quickly oxidizes BH4, potentiating of superoxide production through uncoupled NOS.

While ascorbate is useful in these studies examining simple oxidant stress NO bioavailability mechanisms, it is nonspecific and is capable of increasing NO bioavailability by first acting directly as an antioxidant and secondarily by stabilizing BH4. Therefore, it is impossible to delineate whether the increase in NO-dependent vasodilation in these studies was through an antioxidant effect or through BH4-NOS uncoupling mechanisms. Additional studies examining the relations between arginase, the localization of l-arginine, and BH4 availability are necessary to more fully understand how NOS uncoupling may affect age-related reductions in cutaneous NO-dependent vasodilation.

**Alterations in Vessel Structure**

Maximal cutaneous blood flow decreases linearly as a function of age (50). This age-associated attenuation is observed in both absolute forearm maximal cutaneous vascular conductance assessed with venous occlusion plethysmography and laser-Doppler flowmetry measures of SkBF. The reduction in maximal cutaneous vascular conductance with age presumably reflects structural changes in vessel properties, including, 1 vascular smooth muscle hypertrophy, and 2) a reduction in capillary density associated with flattening of the underside of the epidermis (40, 49). While there are likely many signaling factors stimulating cutaneous microvascular smooth muscle hypertrophy and inward vessel remodeling, obvious candidate pathways include arginase (58), acting through an increase in polyamine and proline synthesis, ROCK, and vasoconstrictor factors produced through COX (76). Chronic activation of these pathways induces functional alterations in microvascular anatomy, which likely contributes to age-related cutaneous vessel structure alterations.

**Platelet Activation**

A series of recent studies in middle-aged humans suggests a novel role for platelets in reflex vasodilation. This proposed novel mechanism emerged from the finding that healthy middle-aged (~58 ± 2 yr) humans taking chronic low-dose (81 mg) aspirin therapy for the primary prevention of atherothrombotic disease displayed a consistent and significant attenuation in reflex cutaneous vasodilation (27). The subjects in this cross-sectional study had been taking low-dose aspirin for greater than 1 yr and displayed a highly attenuated reflex vasodilator response. Platelet inhibition with low-dose aspirin occurs through acetylation of COX-1 in the presystemic (portal) circulation (57), which irreversibly inhibits the production of COX-1 synthesized products, including the potent aggregating agent and vasoconstrictor thromboxane A2. This inhibition occurs for the life of the platelet (~10 days) because they lack a nucleus and, therefore, do not possess the genetic machinery to resynthesize COX. In vascular endothelial cells, significantly higher doses of aspirin (600 mg) are required to fully, but temporarily, inhibit COX which can resynthesize the enzyme in a matter of hours. The most logical mechanism by which low-dose acetylsalicylic acid attenuates reflex vasodilation is that platelets may be activated via COX-1 mechanism and release vasodilating factors, including NO, ATP, ADP, and 5-HT (18, 35, 56), all of which have the potential to stimulate cutaneous vasodilator pathways implicated in reflex vasodilation.

The hypothesis that platelet activation may contribute to reflex vasodilation was further explored utilizing a within-subjects design with two different systemic platelet inhibitors: low-dose aspirin and the specific platelet ADP (P2Y12) receptor antagonist, clopidogrel (Plavix). In this study, specific platelet...
ADP receptor inhibition with clopidogrel significantly attenuated reflex vasodilation to a greater extent than platelet COX-1 inhibition with systemic low-dose aspirin (26). There is in vitro (6, 42) and in vivo (55, 60) evidence for 1) platelet COX-mediated vasodilation in models of neurogenic inflammation, and 2) platelet ADP-receptor-mediated endothelium-dependent vasodilation. Specifically, platelet ADP-receptor stimulation causes the release of dinucleotides stored in platelet dense granule, which potentiates platelet aggregation and induces endothelium-dependent vasodilation (Fig. 3, bottom) (6). The precise mechanism of platelet-vessel wall interactions is complex and not completely understood. However, there is clear evidence for platelets releasing multiple vasodilating factors, some of which are known to contribute to cutaneous reflex vasodilation (5, 36, 51, 74, 75). Further research is necessary to examine the mechanisms of platelet vessel wall interactions underlying this attenuated SkBF response, as well as the potential cardiovascular and thermoregulatory consequences.

PERSPECTIVES

Older aged human skin displays attenuated blood flow responses to both whole body cold and heat stress (24, 31). There are a variety of clinically significant disease states that increase in frequency and severity with aging that may further affect the neurovascular regulation of SkBF. For example, endothelial dysfunction, microvessel wall abnormalities, including inward vessel remodeling, autonomic neuropathy, and/or combinations of these neurovascular impairments are present with atherosclerosis, essential hypertension (28, 29), and diabetes (64, 67), respectively. These modifiers of reflex cutaneous vasodilation and vasoconstriction are a variety of clinically significant disease states that increase in frequency and severity with aging that may further affect the neurovascular regulation of SkBF. For example, endothelial dysfunction, microvessel wall abnormalities, including inward vessel remodeling, autonomic neuropathy, and/or combinations of these neurovascular impairments are present with atherosclerosis, essential hypertension (28, 29), and diabetes (64, 67), respectively. These modifiers of reflex cutaneous vasodilation and vasoconstriction are discussed in detail in an accompanying highlighted topic (7). In addition to these pathological modifiers, common pharmacological therapies used in the treatment of these conditions may also impair key neurovascular signaling mechanisms involved in blood flow regulation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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