Rho kinase-mediated local cold-induced cutaneous vasoconstriction is augmented in aged human skin

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Thompson-Torgerson CS, Holowatz LA, Flavahan NA, Kenney WL. Rho kinase-mediated local cold-induced cutaneous vasoconstriction is augmented in aged human skin. Am J Physiol Heart Circ Physiol 293: H30–H36, 2007. First published April 6, 2007; doi:10.1152/ajpheart.00152.2007.—Cutaneous vasoconstriction (VC), a critical thermoregulatory response to cold, is generally impaired with aging. However, the effects of aging on local cooling-induced VC and its underlying mechanisms are poorly understood. We tested whether aged skin exhibits attenuated localized cold-induced VC and whether Rho kinase-mediated cold-induced VC is augmented with age. Skin blood flow was monitored with laser Doppler flowmetry (LDF) on seven young and seven older subjects. Cutaneous vascular conductance (CVC; LDF/mean arterial pressure) was expressed as percentage change from baseline (%ΔCVCbase). In protocol 1, two forearm skin sites were cooled to six temperatures (31.5–19°C) for 10 min each or two temperatures (29°C, 24°C) for 30 min each, with no age differences in the magnitude of VC. In protocol 2, three forearm skin sites were instrumented for intradermal microdialysis and cooled to 24°C for 40 min. During minutes 1–5, there was no age difference in CVC responses at control sites (young: −45 ± 6% vs. older: −46 ± 3%, P > 0.9). Adrenoceptor antagonism (yohimbine + propranolol) abolished VC in young (to +15 ± 13%, P < 0.05) but only partially inhibited VC in older subjects (to −23 ± 6%, P < 0.05). Rho kinase inhibition plus adrenoceptor antagonism (yohimbine + propranolol + fasudil) abolished VC in both groups. During minutes 35–40, there was no age difference in control (young: −77 ± 4% vs. older: −70 ± 2%, P > 0.3) or adrenoceptor-antagonized responses (young: −61 ± 3% vs. older: −55 ± 2%, P > 0.3); however, Rho kinase inhibition plus adrenoceptor antagonism blocked more VC in older compared with young subjects (−19 ± 11% vs. −35 ± 3%, P < 0.05). Although its magnitude remains unaffected, cold-induced VC becomes less dependent on adrenergic and more dependent on Rho kinase signaling with advancing age.

fasudil; local cooling; vascular function; adrenergic; aging; cutaneous vasoconstriction

Cutaneous vasoconstriction (VC) is the initial thermoregulatory response to cold exposure, effectively minimizing heat loss to the environment through two distinct mechanisms of VC. Whole body cooling evokes sympathetic reflex VC, which is dependent on the release of norepinephrine and cotransmitters from sympathetic adrenergic axon terminals (38–40, 42). In contrast, localized cooling of the cutaneous blood vessels and surrounding tissue engages local (i.e., nonreflex) cold-induced VC that is mediated primarily by norepinephrine at α2-adrenoceptors (9, 12, 14, 25, 28, 32) and Rho kinase (45), along with a proposed rebound constriction via nitric oxide withdrawal (19).

One of the cardiovascular hallmarks of human aging is impaired vascular responsiveness to a stimulus. However, whereas age-associated impairments in reflex cutaneous VC in response to whole body cooling are relatively well understood (26, 27, 34, 42), the effects of advancing age on local cold-induced VC are comparatively poorly characterized. To date, only one study (43) has addressed the effects of aging on VC during local cooling, concluding that there was no age difference. However, that investigation only quantified local cold-induced VC in response to 10 min of local cooling to 24°C, precluding the characterization of possible age differences in VC across a wider physiological spectrum of cooling durations and magnitudes. Given that early- (0–10 min cooling) and late-phase VC (≥20 min cooling) are mediated by different primary mechanisms (25, 32, 45), it is entirely possible that age differences may have arisen with a longer cooling protocol. Thus the existing data do not adequately address the question of whether (or how much) aging affects the pattern and magnitude of local cold-induced VC.

Cutaneous adrenergic desensitization occurs with aging (44), suggesting that the norepinephrine-mediated portion of cold-induced VC may be attenuated in older subjects. Conversely, Rho kinase-mediated VC may be augmented in older humans. Both in vitro and in vivo, Rho kinase is a key intracellular mediator directly involved in VC induced by direct tissue cooling (1, 2, 45). However, Rho kinase activity is augmented in proconstrictor vascular conditions commonly associated with aging, including hypertension, coronary and cerebral vasospasm, erectile dysfunction, and diabetes (6, 16, 23, 24, 29, 33, 46). If Rho kinase-mediated VC is augmented with aging in the absence of disease, it would complement other recent findings suggesting that aging per se is associated with preclinical proconstrictor signaling changes in the vasculature (5, 20, 21).

Accordingly, the purposes of the present study were to systematically characterize responses to localized cooling in young and aged skin and to investigate whether the mechanisms underlying local cold-induced VC change with aging.
We hypothesized that 1) VC is blunted in aged skin as local cooling magnitude and/or duration increases, and 2) norepinephrine-mediated VC diminishes and Rhokinase-mediated VC increases in aged skin.

**METHODS AND MATERIALS**

**Subjects.** Seven young (20–27 yr; 4 men, 3 women) and 7 older (67–74 yr; 3 men, 4 women) subjects all participated in two experimental protocols. All younger women were tested during days 1–7 of the menstrual cycle and none were taking oral contraceptives; all older women were postmenopausal and none were taking hormone replacement therapy. All subjects underwent a standardized medical screening and were healthy, normotensive (blood pressure <130/80 mm Hg) before coming to the laboratory. Approval was obtained from the Institutional Review Board of The Pennsylvania State University. Each subject gave verbal and written informed consent before participation in the study, and all procedures conformed to the standards of the Declaration of Helsinki.

**Protocol 1.** Subjects arrived at the building at 0800 h on the morning of the experiment. After subjects assumed a supine position on the laboratory hospital bed in a temperature-controlled room (~23°C), two forearm skin sites (6.5 cm²) were identified for cooling on the laboratory hospital bed in a temperature-controlled room (~23°C), two forearm skin sites (6.5 cm²) were identified for cooling and collection of skin blood flow data. Skin blood flow was measured using laser Doppler flowmetry (MoorLAB, Moor Instruments) to provide an index of cutaneous red blood cell (RBC) flux. Laser Doppler probes were placed on the skin at each forearm site, and RBC flux data were collected continuously throughout the experiment. Arterial blood pressure was monitored every 20 min via brachial auscultation, and mean arterial pressure (MAP) was calculated as [(1/3 systolic blood pressure) + (2/3 diastolic blood pressure)]. Skin blood flow was converted to cutaneous vascular conductance (CVC; RBC flux/MAP) and expressed as percentage change from baseline CVC values (%ΔCVCbase). Local skin temperature (Tloc) at each site was controlled within ±0.02°C using pelier elements (TecThermo Temperature Controller 1575, Menlo Park, CA) with a small aperture in the center to accommodate the laser Doppler probe. Tloc was clamped at 34°C for baseline data collection for 15 min at each of two sites before the onset of localized cooling. After the baseline period, Tloc at site 1 was randomly cooled to and clamped at each of two temperatures (31.5, 29, 26.5, 24, 21.5, and 19°C) for 10 min with a 15-min 34°C rewarming period between bouts of cooling. At six temperatures (31.5, 29, 26.5, 24, 21.5, and 19°C) for 10 min with a 15-min 34°C rewarming period between bouts of cooling. At site 2, Tloc was randomly cooled to and clamped at each of two temperatures (29°C and 24°C) for 30 min with a 15-min 34°C rewarming period between bouts of cooling. The systematic manipulations of temperature and duration of local cooling in this protocol were designed to reveal whether aging affects the magnitude of, threshold for, or pattern of cold-induced VC.

**Protocol 2.** Subjects arrived at the laboratory at 0800 h on the morning of the experiment and assumed a supine position on the laboratory hospital bed in a temperature-controlled room (~23°C). Three microdialysis fibers (MD-2000, Bioanalytical Systems, West Lafayette, IN) were placed into the ventral surface of the right forearm using sterile technique. For each fiber, a 25-gauge needle was inserted into unanesthetized skin and guided horizontally through the skin such that entry and exit points were ~2 cm apart. The fiber, consisting of a cylindrical 10-mm membrane (320 μm outer diameter, 20 kDa molecular mass cutoff) and connective tubing attached to either end of the membrane, was threaded through the needle. The needle was then withdrawn, leaving the membrane in the skin. After fiber insertion, subjects rested quietly for ~90 min to allow local hyperemia due to insertion trauma to subside, during which time lactated Ringer solution was perfused through all fibers at a rate of 2.0 μl/min (Bee Hive controller and Baby Bee microinfusion pumps, Bioanalytical Systems).

After the insertion trauma resolution period, microdialysis sites were randomly assigned to continuously receive 1) lactated Ringer to act as control, 2) 5 mM yohimbine + 1 mM propranolol (Y + P) to antagonize α₁- and β-adrenergceptors, or 3) Y + P + 3 mM fasudil (Y + P + fasudil) to simultaneously antagonize adrenergceptors and inhibit Rhokinase activity (45). Although yohimbine is traditionally employed as an α₂-adrenergceptor-selective antagonist, it antagonizes both α₁- and α₂-adrenergceptors at the concentration used in this study (18, 38, 39, 42). RBC flux, MAP, CVC, and Tloc were evaluated and controlled in the same manner as described in protocol 1.

After solutions perfused the sites for ~60 min, Tloc was clamped at 34°C for a 15-min baseline period. Norepinephrine (1 μM) (+1 mg/ml l-ascorbate, preservative; 22) was then added to the perfusate through all fibers (in the presence of antagonists) for 4 min to test the integrity of Y + P adrenergceptor antagonism. After a washout period (~30 min, antagonists only), all sites were cooled at a rate of 3°C min⁻¹ and Tloc was clamped at 24°C for 40 min, after which sites were rewarmed back to 34°C.

All drugs were obtained from Sigma-Aldrich (St. Louis, MO), except fasudil, which was obtained from Tocris Bioscience (Ellisville, MO). All drug solutions were mixed just before usage, dissolved in lactated Ringer solution, and sterilized using syringe microfilters (Acrodisc, Pall, Ann Arbor, MI).

**Data collection and analysis.** Data were recorded and stored as 1-min averages using computer software (LabView) and a data acquisition system (National Instruments, Austin, TX). Baseline values for normalization were determined by averaging the last 5 min of baseline data. In both experimental protocols, CVC values recorded during localized cooling were averaged over 5-min intervals for analysis, except for site 1 in protocol 1, where CVC values were averaged during the last 3 min of each 10-min cooling stage. In protocol 2, “early phase” VC was defined as the average of CVC values during the first 5 min of cooling, whereas “late phase” VC was defined as the average of CVC values during the last 5 min of cooling (min 35–40). Student’s t-tests were used to determine significant differences in physical characteristics between young and older subjects. Three-way ANOVA was conducted to detect differences in CVC responses between age groups at difference treatment sites during cooling or norepinephrine administration. Planned-comparison post hoc tests were performed when appropriate to determine where age and treatment differences occurred. Data from young subjects in protocol 2 have been published elsewhere (45) and have been retracted here (Fig. 4) for comparison purposes only. Statistical significance for all analyses was set at α = 0.05. Values are expressed as means ± SE.

**RESULTS**

Subjects in the two age groups were well matched for height, weight, and resting MAP (Table 1). In the older group, n = 7 for data obtained from control sites; however, because of inadequate adrenergceptor antagonism during the norepinephrine test, n = 6 for Y + P and Y + P + fasudil sites. Although mean body mass index was higher (P = 0.01) in older subjects due to a nonsignificant tendency for older subjects to be

<table>
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<th>Variable</th>
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<th>Older</th>
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<tr>
<td>Age, yr</td>
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<td>27 ± 1*</td>
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<td>Height, cm</td>
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<td>Weight, kg</td>
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<td>BMI, kg/m²</td>
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<td>Resting MAP, mmHg</td>
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Values are means ± SE for 7 young and 7 older men (M) and women (F). BMI, body mass index; MAP, mean arterial pressure. *P < 0.05 vs. young. Note: young data have been previously published elsewhere (45) and is presented here for comparison purposes only.
heavier ($P = 0.09$), body mass index was not a significant factor in preliminary statistical analyses, indicating that any observed age differences in local VC were not attributable to differences in body fat or tissue insulation.

Protocol 1. At site 1, there were no age differences in cold-induced VC responses to increasing magnitudes of short-duration local cooling (Fig. 1). At site 2, both the pattern and magnitude of cold-induced VC in response to prolonged cooling were similar throughout the entire protocol in young and older subjects at both 29°C (Fig. 2A) and 24°C (Fig. 2B), with the only difference occurring at $T_{loc} = 29°C$ during the first 5 min of the protocol ($P = 0.04$).

Protocol 2. In representative time-course tracings of cold-induced VC ($T_{loc} = 24°C$ for 40 min) from a young subject (Fig. 3A) and an older subject (Fig. 3B), the relative contributions of the underlying mechanisms mediating VC changed with aging. In the young tracing, Y + P administration attenuated cold-induced VC throughout the cooling protocol, and the addition of fasudil to the adrenoceptor blockade further inhibited VC. In the older tracing, Y + P administration did not inhibit any portion of early-phase cold-induced VC and only mildly attenuated VC as cooling progressed. However, the addition of fasudil to the adrenoceptor blockade virtually abolished VC at all time points.

Mean CVC responses to the prolonged localized cooling protocol exhibited no age differences at control sites, whereas there were significant age differences at both Y + P and Y + P + fasudil sites (Fig. 4). At the control site (Fig. 4A), young and older subjects exhibited similar VC at both early (young: $-45 \pm 6$% vs. older: $-46 \pm 3$% $\Delta CVC_{base}$, $P = 0.9$) and late (young: $-77 \pm 4$% vs. older: $-70 \pm 2$% $\Delta CVC_{base}$, $P = 0.4$) time points (see also Ref. 45). In the presence of combined $\alpha$- and $\beta$-adrenoceptor antagonism (Fig. 4B), older subjects exhibited significant VC during minutes 1–5 ($-23 \pm 6$% $\Delta CVC_{base}$, $P = 0.006$ vs. baseline) in contrast to young subjects, where VC was abolished ($15 \pm 13$% $\Delta CVC_{base}$, $P = 0.001$ vs. baseline; see also Ref. 45); there was no difference in VC observed during minutes 35–40 between young and older subjects (young: $-61 \pm 3$% vs. older: $-55 \pm 2$% $\Delta CVC_{base}$, $P = 0.6$; see also Ref. 45). Combined treatment with adrenoceptor and Rho kinase inhibitors (Fig. 4C) abolished early-phase VC in both young and older subjects with no age difference (young: $-6 \pm 3$% $\Delta CVC_{base}$, $P = 0.4$ vs. baseline; older: $-7 \pm 6$% $\Delta CVC_{base}$, $P = 0.4$ vs. baseline; young vs. older, $P = 0.9$; see also Ref. 45). However, as cooling progressed, there was a significant age difference in late-phase VC at Y + P + fasudil-treated sites, where a greater portion of VC was blocked in older subjects compared with young ($-19 \pm 11$% vs. $-35 \pm 4$% $\Delta CVC_{base}$, $P = 0.04$; see also Ref. 45).

In addition to the cooling protocol, all microdialysis sites also underwent 1 $\mu$M norepinephrine infusion (4 min) to verify the integrity of adrenoceptor antagonism at Y + P and Y + P + fasudil sites. At the control site, both young and older subjects exhibited significant VC response to norepinephrine, although the aged response was significantly attenuated compared with young (young: $-33 \pm 6$% $\Delta CVC_{base}$, $P < 0.0001$ vs. baseline; older: $-13 \pm 4$% $\Delta CVC_{base}$, $P = 0.02$ vs. baseline; young vs. older, $P = 0.0005$; see also Ref. 45). At sites that were treated with adrenergic antagonists, norepinephrine did not induce a significant change in CVC from baseline in either young or older subjects (Y + P, young: $7 \pm 6$% $\Delta CVC_{base}$, young vs. older, $P = 0.2$; see also Ref. 45).
**DISCUSSION**

This study is the latest in a series of investigations that has characterized age-associated changes in cutaneous VC and articulated the altered mechanisms responsible for those changes. Studies utilizing whole body cooling concluded that reflex VC is blunted with age due to an absence of nonadrenergic VC compounded by impaired responsiveness to norepinephrine (42, 44); however, later work utilizing local cooling initially indicated that local VC is paradoxically preserved with age (43), and the present study now confirms and extends those findings to suggest that this maintenance is due to an augmentation of nonadrenergic VC despite impaired responsiveness to norepinephrine. Specifically, the primary findings of the present study indicate that 1) regardless of temperature or duration of local cooling, the magnitude and pattern of cold-induced VC remain unchanged with aging, but that 2) the mechanisms responsible for mediating local cold-induced VC become less norepinephrine dependent and more Rho kinase dependent in aged skin.
Magnitude of cold-induced VC with aging. In protocol 1, the systematic manipulations of temperature and duration of local cooling were designed to reveal whether aging affects the magnitude of, threshold for, or pattern of cold-induced VC. Documented decrements in cutaneous reflex VC function that accompany advancing age (26, 27, 34, 42) suggest that local VC may also undergo a material change with age. However, the results of protocol 1 indicate that the magnitude and pattern of local cold-induced VC remain unchanged in aged skin. Thus, given that the magnitude of local cold-induced VC does not change with aging but that it is dictated in part by adrenergic mechanisms, which undergo desensitization in the cutaneous vasculature with advancing age (44), protocol 2 was conducted to investigate whether the underlying mechanisms of local cold-induced VC in aged skin rely less on adrenergic pathways and more on upregulated compensatory VC pathways, in particular Rho kinase.

Adrenergic mechanisms of cold-induced VC with aging. During the first 10 min of localized skin cooling in vivo in young subjects, immediate and pronounced VC occurs that is dependent on neurally released norepinephrine and is not affected by proximal nerve blockade, suggesting a localized (i.e., nonreflex) mechanism predominantly mediated by norepinephrine of sympathetic origin binding to $\alpha_2$-adrenoceptors (9, 12, 14, 25, 28, 32). However, the effects of aging on the adrenergic portion of cold-induced VC are unknown. In the present study, young and older subjects exhibited the same degree of cold-induced VC at control sites during early phases of cooling; however, whereas early-phase VC in young subjects was completely inhibited by Y + P (45), early-phase VC in older subjects was only inhibited by ~50% at adrenoceptor-antagonized sites (comparing with control responses), revealing a diminished adrenergic contribution to early-phase VC. This finding is supported by the observation that VC was significantly attenuated in aged skin at control sites during thermoneutral norepinephrine administration. These combined results also support and extend previous findings from our laboratory, establishing that adrenergic VC is attenuated during whole body cooling, exogenous norepinephrine administration, and now localized cooling (42–44).

Rho kinase mechanisms of cold-induced VC with aging. Both in vitro and in vivo, Rho kinase is a key intracellular mediator directly involved in local cold-induced VC (1, 2, 45). Rho kinase mediates cold-induced VC through two distinct pathways: 1) inhibition of myosin light chain phosphatase, which passively increases myosin light chain phosphorylation in the absence of Ca$^{2+}$ influx by simply preventing dephosphorylation, leading to functionally increased intracellular sensitivity to exert Ca$^{2+}$; and 2) activation of cold-sensitive $\alpha_2C$-adrenoceptor translocation to the surface of the vascular smooth muscle cell, effectively increasing the adrenoceptor population available to bind norepinephrine (1–3, 10, 15, 17, 36, 37). However, Rho kinase activity is augmented in proconstrictor vascular conditions commonly associated with aging, including hypertension, coronary and cerebral vasospasm, erectile dysfunction, and diabetes (6, 16, 23, 24, 29, 33, 46), raising the question as to whether this augmentation is due solely to pathology or whether it could also be attributed, in part, to the natural aging process.

Our results from protocol 2 confirm that aging alone is associated with augmented Rho kinase-mediated VC. In contrast to young subjects, who exhibited early-phase VC that was abolished by adrenergic blockade, older subjects exhibited early-phase VC that was only partially blocked by adrenergic blockade but completely abolished by the further addition of a Rho kinase inhibitor. These results suggest that the nonadrenergic VC observed during minutes 1–3 at Y + P sites in older subjects was mediated by Rho kinase. As localized skin cooling progressed beyond 10 min, CVC continued to decrease in both age groups at both control and Y + P-treated sites, indicating the addition of nonadrenergic VC mechanism(s) to young vessels and the further contribution of nonadrenergic VC to aged vessels (19, 25, 32, 45). However, whereas there were no CVC differences between young and older subjects at control or Y + P sites, the addition of a Rho kinase inhibitor to the adrenoceptor antagonists during late-phase cooling blocked significantly more late-phase VC in older subjects compared with young, suggesting that the nonadrenergic VC contributions of Rho kinase during late-phase cooling are much more pronounced in older subjects compared with young (45). Both early- and late-phase nonadrenergic VC responses were most likely mediated by the direct effects of Rho kinase on myosin light chain phosphorylation, leading to an increase in the intracellular sensitivity to exert Ca$^{2+}$. These results suggest that augmented Rho kinase-mediated VC may be, at least in part, a function of aging per se rather than the diseases associated with aging; indeed, they complement other recent findings suggesting that aging (in the absence of overt pathology) is associated with preclinical proconstrictor signaling changes in the vasculature (5, 20, 21). These results also suggest that there is at least one other nonadrenergic mechanism that contributes to local cold-induced VC in both young and old, most likely a decrease in nitric oxide synthase activity and/or nitric oxide function (19).

It is surprising that the magnitude of local cold-induced VC is so well maintained throughout old age, especially considering the physiological precedent for compromised vascular function with advancing age. However, the present data suggest that while adrenergic VC may be substantially blunted in aged skin, Rho kinase-mediated VC is augmented sufficiently to compensate for impaired adrenergic VC and results in a net maintenance of local cold-induced VC with aging. It is also possible that, because some of the mechanisms driving cold-induced VC have only recently come to light (19, 45), other as-yet-unknown vascular mediators may also contribute to the maintenance of this response with aging, as well.

There are several plausible mechanisms whereby Rho kinase-mediated VC may be augmented with aging, including two that are dependent on the recently documented decrease in nitric oxide bioavailability associated with advancing age. In young vessels, the nitric oxide pathway downregulates Rho kinase-mediated VC (6, 8, 35), whereas activated RhoA and Rho kinase downregulate several steps in the endothelial nitric oxide synthase pathway (13, 30, 31, 41), effectively maintaining a healthy and necessary balance between dilator and constrictor influences in the vasculature. However, healthy aging is associated with increased vascular arginine activity (5, 21), which competes for the nitric oxide substrate L-arginine and can thus limit functional nitric oxide synthesis. If nitric oxide and downstream cGMP (which deactivates RhoA) are diminished, there may be less appropriate inhibitory regulation of the Rho kinase pathway, leading to augmented Rho kinase...
activity. Aging is also associated with increased production and decreased degradation of reactive oxygen species (ROS) in the skin (7, 11), resulting in a net age-associated increase in oxidative stress in the cutaneous vasculature. ROS, in turn, may enhance Rho kinase-mediated VC indirectly via limitation of nitric oxide bioavailability by ROS-mediated deactivation of nitric oxide, converting it to peroxynitrite before nitric oxide can bind with soluble guanylyl cyclase (4, 20). However, this explanation requires further testing within the context of cold-induced VC to establish its validity. Additionally, ROS may also enhance Rho kinase-mediated VC independent of nitric oxide via direct cold-induced activation of ROS, leading to ROS-mediated activation of Rho kinase activity, as has been demonstrated in in vitro cutaneous vascular preparations (2).

Limitations. Because ROS activity increases in response to cold and activates the RhoA-Rho kinase pathway (3), it is possible that the antioxidant properties of ascorbate (norepinephrine preservative) could have limited Rho kinase activity in the present study. However, given the small dose and short duration of ascorbate delivery (far short of what is required for adequate antioxidant administration in the vasculature), it is unlikely that sufficient ascorbate accumulated before washout to exert significant antioxidant effects on the cutaneous arterioles. Furthermore, if the minute dose of ascorbate did effect ROS and Rho kinase activity, leading to longer-term reduction in ROS activity, its effects would be accounted for at the control site and would not confound the interpretation of data relative to control or other treated sites.

Whereas both early- and late-phase data clearly present evidence supporting the participation of nonadrenergic Rho kinase pathways, the present study does not address whether interactions between adrenergic and Rho kinase pathways (i.e., α2C translocation) also occurred in aged skin. However, whereas Rho kinase significantly mediates VC through both adrenergic and nonadrenergic pathways in young subjects (45), older subjects likely rely less on adrenergic mechanisms to effect VC than their younger counterparts. Adrenergic VC in cutaneous vasculature undergoes significant attenuation with aging due to receptor desensitization (42–44). Those findings suggest that although Rho kinase may stimulate cold-induced α2C-adrenoceptor translocation in aged skin, age-associated impairments in adrenergic signaling compromise the downstream VC response to cold and functionally mask the interaction of the two pathways. Thus adrenergic mechanisms, of necessity, become secondary in aged skin to any compensatory nonadrenergic mechanisms of VC.

In summary, the primary findings of this study indicate that although there is no age difference in the pattern or magnitude of local cold-induced VC after systematic manipulation of both temperature and duration of cooling, the underlying mechanisms that mediate local cold-induced VC are less adrenergic and more Rho kinase dependent in aged skin. This conclusion is supported by both previous and present findings that cutaneous vascular responsiveness to norepinephrine is reduced with aging. The results of this study also support more general findings in the literature suggesting that augmented Rho kinase-mediated VC is associated with age-related pathologies and extend those findings to healthy cutaneous vasculature. Thus whereas augmented Rho kinase-mediated VC may act as a compensatory pathway to preserve local cold-mediated VC with aging, it also serves as an indicator of the increasingly pro-constrictor state of healthy aged vasculature.

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H36  RHO KINASE-MEDIATED VC AUGMENTED IN AGED SKIN