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Age-related impairment of conducted dilation in human coronary arterioles

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Feher A, Broskova Z, Bagi Z. Age-related impairment of conducted dilation in human coronary arterioles. Am J Physiol Heart Circ Physiol 306: H1595–H1601, 2014. First published April 28, 2014; doi:10.1152/ajpheart.00179.2014.—Conducted vasodilation is essential to coordinate vascular resistance along distances to ensure adequate tissue perfusion. We hypothesized that conducted vasodilation of coronary resistance arteries declines with age. Coronary arterioles were dissected from right atrial appendage of patients (n = 27) undergoing cardiac surgery. Arterioles (~100 μm) were cannulated and pressurized (80 mmHg), and developed spontaneous myogenic tone. Conducted vasodilation was initiated by locally administering the endothelium-dependent agonist bradykinin (BK; 100 μM) ejected from a glass micropipette (~3 μm tip opening, positioned in close proximity to the vessel wall). Diameter changes were measured at local and upstream sites (500 and 1,000 μm from the stimulus) with videomicroscopy. Local administration of BK elicited vasodilation, the magnitude of which increased with the duration of stimulus (69 ± 6, 81 ± 6, 90 ± 2%, after 1, 3, and 5 × 100 ms, respectively). BK-induced dilation remained substantial at upstream sites (500 μm: 53 ± 7%; 1,000 μm: 46 ± 9%). The gap junction uncoupler carbenoxolone or 18-o-glycyrrhetinic acid did not affect local responses, but diminished conducted vasodilation. Inhibitors of small/intermediate conductance calcium-activated potassium channels (SKCa/IKCa), apamin and TRAM34, reduced dilations both at local and remote sites. We found that conducted dilation, but not the local response, was significantly reduced in older (~64 yr) patients. The nitric oxide (NO) synthesis inhibitor N-nitro-l-arginine methyl ester did not affect local responses, but markedly reduced conducted dilation in younger (<64 yr) individuals. Collectively, we show that human coronary arterioles exhibit SKCa/IKCa-mediated hyperpolarization spread through gap junctions, which contributes to conducted vasodilation initiated by focal application of BK. We demonstrate that conducted dilation declines with age, likely due to reduced NO availability, which plays a permissive role in propagating longitudinal vasomotor signaling.

human; coronary; resistance artery; spreading vasodilation; aging

BLOOD FLOW IS CONTROLLED BY resistance, and therefore microvascular diameter is the most important parameter governing tissue perfusion. It is known that a localized vasodilator response is not sufficient to create extensive blood flow increase. To ensure adequate myocardial perfusion, to meet the metabolic requirements, vasodilator response must be coordinated along the long distances in an arteriole (28). In aging, vasomotor dysfunction of coronary resistance arteries develops, which can be manifested as a reduced dilator capacity and myocardial flow reserve, even in the absence of manifest coronary atherosclerosis (27). It is possible that an impaired longitudinal vasomotor signaling in the coronary arteriole is the key mechanism responsible for reduced myocardial perfusion in elderly patients with ischemic heart disease.

The conduction of focally evoked vasomotor responses has been established in the microcirculation using animal models (11, 29, 30). To date, no study investigated whether conducted dilation exists in the human coronary microcirculation. It is known that endothelium-derived hyperpolarizing factor (EDHF) contributes critically to the regulation of microvascular tone (5). Indeed, an important aspect of conducted vasomotor responses is the electrotonic spread of hyperpolarizing current through the vascular wall (12, 14). In this process gap junctions, physical contacts between vascular cells, plays a major role to enable cell-cell communication and hyperpolarization spread to allow synchronous diameter changes of resistance arteries (10, 12). A hyperpolarizing current may pass between endothelial and smooth muscle cells via myoendothelial gap junctions (35), but evidence also indicate that myoendothelial coupling is weak in the murine skeletal muscle microcirculation in vivo (32).

The crucial role for endothelial communication in contributing to conducted vasodilation has been demonstrated earlier (13, 32). Behringer et al. (2) have shown that aging impairs electrical conduction along endothelial cell tubes obtained from resistance arteries of the 24- to 26-mo-old mice. In old (19 to 20 mo) mice, Bearden et al. (1) found that conducted vasodilation is reduced in the skeletal muscle microcirculation, in vivo, compared with younger (12–14 mo) mice. These data indicate that aging impairs conducted vasodilation in the skeletal muscle circulation and demonstrate a key role in dysfunctional microvascular endothelium in this process. Only limited data are available regarding gap junction-mediated endothelial signaling in coronary resistance arteries (15), and to date it has not been determined whether conducted dilation is altered in coronary resistance arteries of elderly patients.

Therefore, in this study, with the use of small vessel pressure myography and videomicroscopy, conducted vasodilation of human coronary arterioles was investigated upon focal application of endothelium-dependent dilator bradykinin. We hypothesized that EDHF-mediated hyperpolarization and hyperpolarization spread through gap junctions contribute to conducted vasodilation of coronary resistance arteries and that the magnitude of the conducted coronary artery dilation declines with age.

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MATERIALS AND METHODS

Patients. All protocols were performed as approved by the Institutional Review Board at Georgia Regents University. Consecutive patients undergoing heart surgery were enrolled in this study. Patients were divided into younger (<64 yr) and older (≥64 yr) groups. When conducted responses were evaluated, coronary arteriolar diameter changes were also plotted against the patients’ age.

Small artery pressure myography and videomicroscopy of the human coronary arteriole. Videomicroscopy of isolated human resistance arteries was performed as previously described (6, 19, 34). Briefly, human coronary arterioles were dissected from right atrial appendages obtained from patients at the time of cardiac surgery. After dissection, arterioles (~80 μm internal diameter and at least 1,500 μm in length) were cannulated and pressurized (80 mmHg) and changes in diameter were continuously measured with a videocapnograph.

Assessment of conducted dilation in the human coronary arteriole. Conducted dilation was initiated by a locally confined stimulation of an arteriole, similar to as described earlier (32). Briefly, with the use of a pneumatic injector (30–50 kPa; pressure for 1, 3, and 5 × 100 ms in duration), bradykinin (100 μM) was ejected from a glass micropipette (tip opening of 3 μm) positioned in close proximity to the vessel wall. Superfusion was positioned to wash away the agonist from the site of observation. After diameter responses were obtained at local sites and the vessels returned to their initial diameter, the same stimuli were applied without repositioning the pipette but measuring arterial dilations at remote sites, 500 and 1,000 μm from the stimulating pipette.

To investigate the underlying mechanisms responsible for conducted vasodilation, diameter changes to focally applied bradykinin were measured after incubation of the vessels with nitric oxide (NO) synthase inhibitor Nω-nitro-l-arginine methyl ester (l-NAME; 200 μmol/l, for 30 min), the cyclooxygenase inhibitor indomethacin (10 μmol/l, for 30 min), or inhibitors of small/intermediate conductance calcium-activated potassium channels (SKCa; apamin, 100 nmol/l, for 30 min; I KCa, TRAM34, 10 μmol/l, for 30 min). To demonstrate a role of gap junctions in mediating conducted vasodilation, diameter changes were obtained in the presence of gap junction uncoupler carbenoxolone (100 μmol/l, for 30 min) or 18-α-glycyrrhetinic acid (10 μmol/l, for 30 min).

Data analysis and statistics. Bradykinin-induced arteriolar dilations are expressed as changes in diameter as a percentage of the maximal dilation, defined as the passive diameter of the vessel at 80 mmHg intraluminal pressure in a calcium-free medium. Unless otherwise indicated, data are expressed as scattered dot plots or box-and-whisker plots, in which the minimum, the 25th percentile, the median, the 75th percentile, and the maximum are presented. Kolmogorov-Smirnov test was performed for normality, and data were then analyzed with Pearson correlation and also with linear regression. Statistical analyses were performed using GraphPad Prism software by two-way ANOVA followed by Tukey post hoc test. P < 0.05 was considered statistically significant.

RESULTS

Conducted dilation in the human coronary arteriole. In the first series of experiments, similar to our previous studies (3, 6, 19, 34), human coronary arterioles were dissected from right atrial appendages of patients undergoing cardiac surgery. Patient characteristics are summarized in Table 1. The isolated coronary arterioles developed a spontaneous tone; the passive diameter in calcium-free solution was 124 ± 8 μm, and the active diameter in calcium containing solution was 72 ± 4 μm.

Conducted dilation was initiated by a locally confined stimulation with bradykinin (100 μM, consecutive stimuli with 1, 3, and 5 × 100 ms in duration) ejected from a glass micropipette positioned in close proximity to the vessel wall. Representative traces show that locally initiated dilation to bradykinin was substantial, which also remained significant at 500 and 1,000 μm upstream from the stimulation site (Fig. 1A, left). Increasing the duration of bradykinin stimuli (i.e., increasing the concentration of locally administered bradykinin) resulted in gradually increased local and conducted vasodilation, and the magnitude of the conducted response was significantly reduced at upstream sites (Fig. 1B). We found that two different gap junction uncoupling pharmacological probes, carbenoxolone (Fig. 1A, middle, and 1C) and 18-α-glycyrrhetinic acid (Fig. 1A, right, and 1D), diminished bradykinin-induced conducted dilation at remote sites, whereas they had no effect on the magnitude of local response. In time control experiments the second administration and stimulation with bradykinin did not show any significant ‘rundown’ or desensitization, i.e., the magnitude of dilations remained the same both at local and remote sites upon repeated drug applications (Fig. 1E).

Age-related impairment of conducted coronary dilation. To test the hypothesis that aging impairs conducted vasodilation, patients were divided into younger (<64 yr) and older (≥64 yr) groups, and vascular responses were compared. Interestingly, coronary arterioles from older patients exhibited a somewhat increased dilations in response to bradykinin at local site (Fig. 2A). Importantly, we found a significant reduction in the magnitude of the conducted responses, as measured by the dilation to bradykinin at remote sites (Fig. 2, C and E). Correspondingly, we observed significant, negative correlations between bradykinin-induced conducted, but not local, dilations and patients’ age (Fig. 2, B, D, and F).

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<th>Table 1. Patient demographics, diseases, and medications</th>
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Values are means ± SD; n = patients’ number.
Contribution of EDHF and NO to conducted coronary dilation induced by bradykinin. It is known that bradykinin may activate various vasodilator signaling pathways in the coronary resistance artery, involving but not limited to EDHF, NO, and prostanoids (17). To dissect the molecular mechanisms responsible for local and conducted vasodilation, bradykinin-induced responses were obtained after inhibition of EDHF-, NO-, and prostanoid-mediated signaling. We found that in the presence of apamin and TRAM34, inhibitors of SKCa/IKCa channels, both local and conducted dilations to bradykinin were markedly reduced (Fig. 1F). When experiments were performed in 30 mM K⁺ solution, i.e., to inhibit EDHF, local and conducted vasodilation to bradykinin were abolished (Fig. 1G). Local and conducted dilation of coronary arterioles were also assessed before and after incubation with the cyclooxygenase inhibitor indomethacin. We have found that neither local nor conducted responses were affected by indomethacin (before indomethacin: 51 ± 7, 30 ± 5, 18 ± 2%; after indomethacin: 50 ± 3, 29 ± 6, 18 ± 6% at local, 500 and 1,000 µm, respectively, N = 3; data are means ± SE, nonsignificant). These data indicated no or only a minor role of prostanoids in bradykinin-induced local and conducted coronary responses.

Moreover, we found that inhibition of NO synthesis did not affect local responses (Fig. 3, A and B), whereas l-NAME significantly reduced conducted dilations to bradykinin. It is important to note that the magnitude of the inhibition by l-NAME, i.e., the l-NAME-inhibited portion of the conducted dilation, was significantly greater and more prominent in younger patients (Fig. 3, C and E) than in older individuals (Fig. 3, D and F).

DISCUSSION

Vasomotor responses in the coronary microcirculation match blood flow to metabolic requirements. The role of intercellular coupling in this process has not yet been established in humans and may act to amplify changes in coronary vascular resistance. Here we demonstrate that conducted vasodilation occurs in the human coronary arteriole and that the magnitude of conducted response declines with age. Key data within this study show that 1) focally induced vasodilation to
bradykinin is primarily mediated by activation of SKCa/IKCa channels and subsequent, gap junction-dependent hyperpolarization spread and that 2) the conducted dilation is facilitated by endogenous NO. We propose that 3) a reduced NO bioavailability contributes to age-related decline of conducted vasodilation in the human coronary arteriole.

Conducted dilation in the human coronary arteriole. The conducted vasodilation has been established in animal models using in vivo or ex vivo vessel preparations (11, 29, 30). Our present study translates these previous observations to humans, and also extends previous findings obtained in various vascular beds toward the coronary resistance artery. Similar to that of observations in rodent vessels (12, 15), we found that an important aspect of conducted vasodilation is the hyperpolarization spread through gap junctions along the microvascular wall. To furnish evidence for this scenario our functional experiments show that after the SKCa/IKCa channels (known
coronary flow reserve is diminished in elderly patients, even in the absence of significant stenosis of epicardial coronary arteries (27), a condition commonly associated with coronary microvascular dysfunction. Collectively, previous studies indicated that aging is associated with reduced dilator capacity of both conduit and resistance arteries. To ensure adequate myocardial perfusion, a vasodilator response must be coordinated along the long distances in the feed and resistance coronary artery. Therefore, it is plausible that an impaired longitudinal signaling in the coronary artery wall is one of the key mechanisms responsible for impaired myocardial perfusion in elderly patients.

Coronary microvascular dysfunction in elderly patients. Aging is associated with an increased incidence of heart disease and stroke accounting for significant morbidity and mortality in this growing population (20). Emerging evidence indicates that one of the key manifestations of age-related ischemic heart disease is the development of coronary microvascular dysfunction, a pathology, which may predispose elderly patients to periods of myocardial ischemia. The underlying mechanism(s) of aging-associated coronary microvascular disease remain poorly understood, so that effective preventive therapeutic strategies cannot be adopted. Older adults exhibit endothelial dysfunction, which is characterized by impaired relaxation of the brachial artery (33). In addition,
mediators of EDHF and hyperpolarization spread) (2) were inhibited or in the presence of 30 mM K+ solution (known to inhibit EDHF response), the conducted coronary dilation to bradykinin is abolished. Given that previous studies found an impaired conducted vasodilation in the skeletal muscle of rodent models of aging (1, 2), in this study we also determined whether the conducted dilation is altered in coronary resistance arteries of older patients. We found a significant reduction in conducted dilation to bradykinin in older (≥64 yr) patients compared with younger individuals (<64 yr). Correspondingly, we observed significant, negative correlations between the magnitude of conducted vasodilation and the patients’ age. We found no significant correlations in the local responses, and, somewhat unexpected, older patients exhibited small but significantly enhanced local dilations to focal bradykinin applications.

The exact mechanisms responsible for the reduced conducted vasodilation, and maintained or even enhanced local coronary dilator response to bradykinin remains unclear. Early reports show that ACh-induced dilation is reduced in norepinephrine preconstricted mesenteric (18) and coronary (9) arteries from aged rats. Recently, Chennupati et al. (7) have found an essentially maintained ACh-induced, but significantly reduced SKCa/IKCa channel activator, NS309-dependent relaxation, in preconstricted saphenous arteries of 64-wk-old male mice. In contrast, and in relation to the conducted vasomotor response, Behringer et al. (2) have shown that either direct stimulation with NS309 or indirect stimulation with ACh, activation of SKCa/IKCa channels to produce hyperpolarization and hyperpolarization spread, is preserved in endothelial tubes of old mice. The apparent controversy in these studies has yet to be solved.

Another important aspect of the conducted vasomotor response is the electrotonic spread of hyperpolarizing current through gap junctions, which enables cell–cell communication and hyperpolarization spread to initiate synchronized diameter changes (12, 35). Several types of connexin (Cx) have been identified in vasculature: Cx40, Cx43, and Cx45 is expressed in smooth muscle cells, whereas Cx37, Cx40, and Cx43 in endothelial cells (35). Skeletal muscle arterioles of Cx40-deficient mice exhibit a strong impairment of the conducted dilation initiated by bradykinin compared with wild-type mice (11). There is great paucity in the literature in evaluating the potential impact of aging on the expression and function of vascular smooth muscle cells, whereas activation of SKCa/IKCa channels via enhancing the electrical gradient for Ca2+ influx promotes endothelial NO synthase activation (31). In view of this interaction and because age-related endothelial dysfunction is primarily characterized by reduced availability of NO (8, 9, 36), in this study we also sought to determine the role of NO in contributing to conduced dilation in the human coronary arteriole. Similar to previous observations obtained in human coronary arteriole (16, 25), inhibition of NO synthesis did not significantly reduce local dilation to bradykinin, suggesting minor, if any, involvement of NO in this response. Intriguingly, inhibition of NO synthesis markedly reduced conducted dilations to bradykinin, with a much greater extent in younger individuals than in elderly patients, whereas responses were not affected by inhibition of prostanooid synthesis. This inhibitory effect by l-NAME was unexpected in this study. An earlier study has shown that local release of NO or exogenous NO donor applications does not contribute to the conducted vasomotor response in the hamster cremaster muscle arteriole (22). A later study has found that NO specifically reduces the permeability of Cx37-containing gap junctions (23). Because in the present study we demonstrate that elevation of extracellular K+ abrogated the conducted response in human coronary arterioles, it is possible that NO may amplify longitudinal arteriolar signaling, which is independent of mod-

Fig. 4. Schematic drawing illustrates proposed underlying mechanisms of conducted dilation in isolated human coronary arterioles in young (left) vs. aging (right) patients. Human coronary arterioles exhibit small/intermediate conductance calcium-activated potassium channel (SKCa/IKCa)-mediated, endothelium-dependent hyperpolarization factor (EDHF)-dependent dilations induced by focal administration of bradykinin (BK). Endothelial hyperpolarization then may spread via myoendothelial gap junction (MEJ) toward, and also via gap junctions (GJ) between, vascular smooth muscle cells to induce conducted vasodilation. We propose that the reduced availability of nitric oxide (NO), which seems to play a permissive role in SKCa/IKCa-mediated, endothelium-dependent hyperpolarization, results in an impairment of conducted vasodilation in aging. BKR, bradykinin receptor.
IKCa channels often have off-target effects. Data obtained with previously observed effect of L-NAME on membrane potential to be elucidated. Another example of off-target effects is the cellular versus heterocellular hyperpolarization spread has yet myoendothelial gap junctions; thus the involvement of homocarbenoxolone, are not distinguishing between endothelial and known that commonly used gap junction blockers, such as effect by L-NAME may explain the apparent differences in limits bradykinin-induced hyperpolarization spread, which is independent of NO synthase/NO/soluble guanylate cyclase pathway (26). A study of vascular smooth muscle cells, which is independent of NO deactivation of calcium-dependent potassium channels in vascular smooth muscle. Nature 368: 850–853, 1994.

Furthermore, pharmacological probes for inhibition of SKCa/IKCa channels often have off-target effects. Data obtained with these probes need to be interpreted with caution. It is also known that commonly used gap junction blockers, such as carbonoxolone, are not distinguishing between endothelial and myoendothelial gap junctions; thus the involvement of homocellular versus heterocellular hyperpolarization spread has yet to be elucidated. Another example of off-target effects is the previously observed effect of L-NAME on membrane potential of vascular smooth muscle cells, which is independent of NO synthase/NO/soluble guanylate cyclase pathway (26). A study by Murphy et al. (26) has shown that in rat cremaster muscle arteriole L-NAME caused constriction with a small (3 to 4 mV) depolarization of the arteriolar smooth muscle (26). It is possible that even a small depolarizing current by L-NAME limits bradykinin-induced hyperpolarization spread, which may result in a reduced conducted vasodilation. How this effect by L-NAME may explain the apparent differences in conducted coronary responses observed between younger and older individuals remains elusive. Also, further mechanistic studies are needed to explore underlying mechanisms responsible for reduced NO bioavailability in elderly patients.

Summary and conclusions. In summary, we show that human coronary arterioles exhibit SKCa/IKCa-mediated hyperpolarization spread through gap junctions, which contributes to conducted vasodilatation initiated by focal application of bradykinin. We demonstrate that conducted dilation declines with age, likely due to the reduced availability of NO, which seems to play a permissive role in propagating longitudinal vasomotor signaling in the human coronary arteriole (Fig. 4). We propose that an impaired longitudinal signaling in the coronary resistance artery wall is one of the key mechanisms responsible for impaired myocardial perfusion in elderly patients.

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GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: A.F., Z. Broskova, and Z. Bagi performed experiments; A.F., Z. Broskova, and Z. Bagi analyzed data; A.F. and Z. Bagi interpreted results of experiments; A.F., Z. Broskova, and Z. Bagi prepared figures; A.F. and Z. Bagi drafted manuscript; A.F., Z. Broskova, and Z. Bagi approved final version of manuscript; Z. Bagi conception and design of research; Z. Bagi edited and revised manuscript.

REFERENCES

17. Fleming I, Michaelis UR, Bredenkotter D, Fisslthaler B, Dehghani F, Brandes RP, Busse R. Endothelium-derived hyperpolarizing factor syn-


