Physiology of Aging

Selected Contribution: Effects of aging on cerebrovascular tone and \([\text{Ca}^{2+}]_i\)

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Submitted 27 March 2003; accepted in final form 17 June 2003.

Geary, Greg G., and John N. Buchholz. Selected Contribution: Effects of aging on cerebrovascular tone and \([\text{Ca}^{2+}]_i\). J Appl Physiol 95: 1746–1754, 2003. First published June 20, 2003; 10.1152/japplphysiol.00275.2003.—The lower limits of cerebral blood flow autoregulation shift toward high pressures in aged compared with young rats. Intraluminal pressure stimulates contractile mechanisms in cerebral arteries that might, in part, cause an age-dependent shift in autoregulation. The present project tested two hypotheses. First, cerebral artery tone is greater in isolated arteries from aged compared with mature adult rats. Second, aging decreases the modulatory effect of endothelium-derived nitric oxide (NO) and increases vascular smooth muscle \(\text{Ca}^{2+}\) sensitivity. Isolated segments of middle cerebral arteries from male 6-, 12-, 20-, and 24-mo-old Fischer 344 rats were cannulated and loaded with fura-2. Diameter and \(\text{Ca}^{2+}\) responses to increasing pressure were measured in HEPES, during NO synthase inhibition \([N^\text{G}-\text{nitro-L-arginine methyl ester (L-NAME)}],\) and after removal of the endothelium. Cerebral artery tone (with endothelium) increased with age. Only at the lowest pressure (20 and 40 mmHg) was intracellular \(\text{Ca}^{2+}\) concentration \([\text{Ca}^{2+}]_i\) greater in arteries from 24-mo-old rats compared with the other age groups. \(\text{L-NAME}\)-sensitive constriction increased significantly in arteries from 6- to 20-mo-old rats but declined significantly thereafter in arteries from 24-mo-old rats. \([\text{Ca}^{2+}]_i\) was less in arteries from 24-mo-old rats compared with the other groups after treatment with \(\text{L-NAME}\). Another endothelial-derived factor, insensitive to \(\text{L-NAME}\), also decreased significantly with age. For example, at 60 mmHg, the \(\text{L-NAME}\)-insensitive constriction decreased from 47 ± 10, 42 ± 5, 21 ± 2, and 3 ± 1 \(\mu\)m in 6-, 12-, 20-, and 24-mo-old rats, respectively. Our data suggest that aging alters smooth muscle \(\text{Ca}^{2+}\) sensitivity through endothelial-derived NO synthase-sensitive and -insensitive mechanisms. The combined effect of greater cerebral artery tone with less endothelium-dependent modulation may in part contribute to the age-dependent shift in cerebral blood flow autoregulation.

endothelium; nitric oxide; calcium sensitivity; rat; pressure; intracellular calcium concentration

Cerebral blood flow (CBF) remains relatively constant over a wide range of arterial pressure (27). Stability of CBF (termed autoregulation) is in part, accomplished via constriction of cerebral arteries as pressure rises and dilation as pressure declines (25). The theoretical lower and upper limits of CBF autoregulation are ~50–60 and 150–160 mmHg, respectively (27). The effects of aging on CBF autoregulation have been examined in healthy, normotensive, and hypertensive rats. These aging studies show that the lower limits of CBF autoregulation shifts toward high pressures in aged compared with young rats without an apparent effect on resting regional blood flow (13, 26, 46).

Alterations to the lower limit of CBF autoregulation with aging might relate to numerous age-dependent anatomic and functional alterations to the cerebral vasculature. Aging is known to cause vascular stiffening by decreasing distensibility (less smooth muscle and elastin) and increasing less distensible (greater collagen and basement membrane) components of the vessel wall (17, 33). Combined, these normal consequences of vascular aging may influence, either individually or in combination, the lower limits of autoregulation in healthy animals (13, 26).

Vascular function changes are also known to change with advancing age. For example, contractility of cerebral arteries to serotonin is enhanced with age, resulting in greater reduction of CBF in senescent (24–27 mo) compared with adult (9–12 mo) rats (18). In addition to vascular smooth muscle cell changes with age, anatomic and functional changes occur to the endothelial layer of cells. Thinning of the vascular endothelium has been noted with age (33), as well as diminished endothelium-dependent relaxations to ACh and bradykinin in rat cerebral arteries (33). What is currently undetermined from previous aging studies is whether the response of cerebral arteries to pressure (myogenic response) (6) is altered in aged compared with mature adult rats.

Cerebral arteries possess “tone” or a “regional blood flow reserve” necessary for establishment of the lower and upper limits of CBF autoregulation. By small changes in arterial tone, this blood flow reserve is adjusted to meet neuronal or metabolic needs (27). In vitro, cerebral artery tone occurs through excitatory...
pathways requiring vascular smooth muscle cell membrane depolarization and increased cytosolic Ca\(^{2+}\) (6). Pressure also has a stimulatory effect on vascular endothelium, although typically believed unnecessary for development of tone (6). The vascular endothelium responds to pressure by modulating the magnitude of tone through release of vasodilatory [nitric oxide (NO), prostacyclin (PGI\(_2\)], and an endothelial-derived hyperpolarizing factor (EDHF)] and contractile substances (endothelin-1) (11).

To date, no study has addressed the effect of healthy aging on cerebral artery tone, artery wall Ca\(^{2+}\), and the endothelial-dependent response to pressure in isolated, rat cerebral arteries. The present project tested the hypothesis that greater tone in arteries from aged compared with adult rats occurs because of a change in the modulatory effect of endothelium-derived NO and vascular smooth muscle Ca\(^{2+}\) sensitivity. Young adult (6 mo), mature adult (12 and 20 mo), and old (24 mo) Fischer 344 rats were chosen for the present study because of their well-documented life span and sexual development (9). In addition, this strain of rat is relatively free from atherosclerotic lesions and hypertension (9). Thus the Fischer 344 is a model of relatively healthy vascular aging in which the impact of age on cerebral artery tone can be successfully studied.

METHODS

General preparation. The animal research committee of Loma Linda University approved all procedures. Male Fischer 344 rats (Jackson Laboratory, Bar Harbor, ME) were housed under a 12:12-h light-dark cycle with food and water available ad libitum. Young adult (6 mo), mature adult (12 and 20 mo), and old (24 mo) Fischer 344 rats were chosen for the present study because of their well-documented life span and sexual development (9). In addition, this strain of rat is relatively free from atherosclerotic lesions and hypertension (5, 9, 12, 26). Thus the Fischer 344 is a model of relatively healthy vascular aging in which the impact of age on cerebral artery tone can be successfully studied.

Endothelium removal. Endothelium removal was accomplished by perfusing 1 ml of air through the artery lumen. Successful removal of the endothelium was verified by the absence of a vasodilator response to 10 \(\mu\)M ADP, an endothelium-dependent vasodilator in this preparation (16).

Measurement of smooth muscle Ca\(^{2+}\) in pressurized arteries. To obtain measurements of intracellular concentration Ca\(^{2+}\) ([Ca\(^{2+}\)\(]_i\)] in vascular smooth muscle cells, cannulated arteries were loaded with fura-2-AM, a Ca\(^{2+}\)-sensitive fluorescent dye (47). Fura 2 (10 \(\mu\)M), from 1 mM stock dissolved in DMSO, was premixed with 1% Pluronic (10% solution in H\(_2\)O) and then diluted in PSS to yield a final fura-2-AM concentration of 1 \(\mu\)M. The cannulated middle cerebral artery segment was incubated in the fura-2-AM-PSS loading solution at room temperature in the dark for 18 min. Fura 2-loaded arteries were then washed with PSS, and bath temperature was increased to 37°C. Fura-2 fluorescence was measured by using a photomultiplier system (IonOptix) in which background-corrected ratios (collected once every 10 min) were subtracted from the 510-nm emission from arteries alternatively excited at 340 and 380 nm (fura 2 ratio; \(R_{Ca}\)). The sampling rate was 3 Hz. The 380-nm signals among artery groups were similar (6 mo, 0.245 ± 0.007; 12 mo, 0.249 ± 0.01; 20 mo, 0.246 ± 0.01; and 24 mo, 0.257 ± 0.007), suggesting that fura-2 loading was not affected by age (39, 42). The experimental protocol started after a 60-min equilibration period at 60 mmHg.

Experimental protocol. Percent changes in smooth muscle Ca\(^{2+}\) (\(R_{Ca}\)) and artery diameter in response to increases in transmural pressure (10–80 mmHg), in the absence and presence of a NO synthase (NOS) inhibitor, and after endothelium removal were determined in each artery from the four different age groups. As previously described (16), the first series of pressure steps was completed in PSS and the second series was completed in the presence of N\(^{6}\)-nitro-L-arginine methyl ester (L-NAME; 100 \(\mu\)M). A third series of pressure steps was completed following endothelium removal. A final series of pressure steps was conducted in Ca\(^{2+}\)-free PSS with EGTA (3 mM) to determine passive diameters and minimum Ca\(^{2+}\) levels. L-NAME and EGTA were added to the organ chamber 20 min before commencement of pressure steps, and each pressure step was maintained for 5–10 min to allow vessel diameter to stabilize before measurement.

Chemicals. Pluronic and fura-2-AM were purchased from Molecular Probes (Eugene, OR). All other drugs were purchased from Sigma Chemical (St. Louis, MO) and were added, individually or in combination, to the organ chamber in their final optimally effective concentrations.

Data analysis and statistics. Changes in \(R_{Ca}\) in response to increasing pressure were normalized to the \(R_{Ca}\) at 10 mmHg in PSS. Fluorescent ratios (340 nm/380 nm) at 10 mmHg were as follows: 6 mo, 0.222 ± 0.008; 12 mo, 0.230 ± 0.003; 20 mo, 0.239 ± 0.009; and 24 mo, 0.225 ± 0.012. Contractile effects of L-NAME were determined by subtracting artery diameter after drug treatment from artery diameter before drug treatment at any given pressure. Cerebral artery tone (%) was determined in endothelium-intact arteries by subtracting the diameter at any given pressure from the maximum passive diameter (obtained in 0 Ca\(^{2+}\) with 3 mM EDTA; 80 mmHg) and dividing the difference by the maximum passive diameter. Similarly, cerebral artery tone (%) was determined in endothelium-denuded arteries by subtracting the diameter at any given pressure from the maximum passive diameter (0 Ca\(^{2+}\) with 3 mM EGTA; 80 mmHg) and dividing the difference by the maximum passive diameter. Data are expressed as means ± SE. Statistical significance was determined by using ANOVA with Scheffé’s test for post hoc comparisons. Statistical significance implies \(P < 0.05\) unless otherwise stated.

RESULTS

Animal body weights. Rat body weights did not significantly change with advancing age and averaged 373 ± 7 g (6 mo), 417 ± 6 g (12 mo), 433 ± 5 g (20 mo), and 392 ± 7 g (24 mo).
Effects of pressure on diameter and tone. In the absence of extracellular Ca\(^{2+}\) (+EGTA, 3 mM) in the bath solution, artery diameters increased in response to elevation of transmural pressure (20–80 mmHg, passive response; Fig. 1 A–D). Maximal passive diameters (80 mmHg) of arteries from 6- (263 ± 5 \(\mu\)m), 12- (263 ± 3 \(\mu\)m), and 20-mo-old rats (277 ± 9 \(\mu\)m) were significantly larger than 24-mo-old rats (251 ± 3 \(\mu\)m) \((P < 0.05, \text{ANOVA})\). In Ca\(^{2+}\)−containing PSS, diameters of endothelium-intact arteries from all groups of rats were significantly smaller than their passive responses (Fig. 1, A–D). To determine whether age affects the magnitude of constriction, we calculated tone (with endothelium) or the percent diameter difference between maximum passive diameter and PSS responses (Fig. 1E). Although all groups of arteries in Fig. 1E developed tone, the magnitude of this response was significantly greater in arteries from 24-mo-old rats compared with 6- and 12-mo-old rats.

Ca\(^{2+}\) responses to pressure. In the absence of extracellular Ca\(^{2+}\) in the bath solution, increasing pressure (20 and 80 mmHg) did not affect R\(_{Ca}\) (%) from any group of arteries (data not shown). A trace of diameter (Fig. 2A) and R\(_{Ca}\) (340 nm/380 nm; Fig. 2B) in arteries from 24-mo-old rats is shown at 40 mmHg and after a single pressure step to 60 mmHg (Fig. 2C). At both 20 and 40 mmHg in Ca\(^{2+}\)−containing PSS, R\(_{Ca}\) from 24-mo-old rats was significantly greater than the other groups (Fig. 2D; \(P < 0.05, \text{ANOVA}\)). However, at higher pressure (60 and 80 mmHg), R\(_{Ca}\) was not significantly different among groups (Fig. 2D).

Effects of L-NAME on diameter and R\(_{Ca}\). To determine whether age affects NO-dependent modulation of diameter, we measured diameter in response to pressure in the presence of the NOS inhibitor L-NAME (100 \(\mu\)M). L-NAME treatment significantly reduced artery diameter from all groups compared with diameter responses in PSS (Fig. 3, A–D; \(P < 0.05, \text{ANOVA}\)). To determine whether age affects the contractile effects of L-NAME, we calculated L-NAME-sensitive constriction (Fig. 3E). Although age clearly impacted L-NAME-sensitive constriction in arteries from 6- to 20-mo-old rats (20–80 mmHg), constriction decreased significantly in arteries from 24-mo-old rats compared with response of 20-mo-old rats (\(P < 0.05, \text{ANOVA}\)). L-NAME-sensitive constriction was similar between 24-mo-old rats and arteries from 6- and 12-mo-old rats.

In arteries from 6-, 12-, and 20-mo-old rats, L-NAME treatment significantly increased R\(_{Ca}\) responses compared with responses in PSS (Fig. 4, A and B; \(P < 0.05, \text{ANOVA}\)). The effect of L-NAME on R\(_{Ca}\) was most evident at the lowest pressures (40 mmHg) where R\(_{Ca}\) increased nearly fourfold in these arteries. L-NAME treatment did not significantly affect the R\(_{Ca}\) responses in arteries from 24-mo-old rats compared with their response in PSS. When R\(_{Ca}\) at any pressure was compared between groups (L-NAME treated), R\(_{Ca}\) from 24-mo-old rats were significantly less than responses from 6- and 12-mo-old rats.

Effects of endothelium damage on diameter and R\(_{Ca}\). To determine whether additional endothelium-derived modulatory substances (insensitive to NOS inhibition) were present in rat cerebral arteries and affected by advancing age, we determined the effect of pressure on artery diameter after disruption of the endothelium. To verify efficient endothelium denudation, we determined responses to the endothelium-dependent dilator ADP (10 \(\mu\)M). In endothelium-intact arteries, the magnitudes of vasodilation to ADP were significantly greater in arteries from 6- (27 ± 8\%, \(n = 11\)) and 12-mo-old rats (21 ± 7\%, \(n = 11\)) compared with either 20- (9 ± 1\%, \(n = 7\)) or 24-mo-old rats (11 ± 2\%, \(n = 6\)).
endothelium removal, ADP did not produce a significant dilation in any of the groups.

In 6-, 12-, and 20-mo-old animals, endothelium disruption (Endo) significantly constricted artery diameter compared with responses in L-NAME (Fig. 5, A–C; \( P < 0.05 \), ANOVA). Unlike the responses to pressure in arteries from the younger animals, arteries from 24-mo-old rats were not significantly affected by Endo compared with their response in L-NAME (Fig. 5D).

Calculation of the diameter difference between L-NAME treatment and Endo (L-NAME-insensitive constriction; Fig. 5E) showed that L-NAME-insensitive constriction diminished significantly in arteries from 20-old rats compared with the 6- and 12-mo-old responses. Additionally, L-NAME-insensitive constriction of arteries from the oldest age group (24 mo) was significantly smaller than L-NAME-insensitive constriction in arteries from 20-mo-old rats (\( P < 0.05 \), ANOVA).

To determine whether age affects the contractile function of the artery after endothelium disruption, we calculated tone (Endo) or the percent diameter difference between maximum passive diameter and Endo (Fig. 6A). After endothelium disruption, arteries from 6-, 12-, and 20-mo-old rats developed similar and stable tone in response to pressure. However, in endothelium-denuded arteries from the 24-mo-old rats, cerebral artery tone declined significantly compared with the other groups (\( P < 0.05 \), ANOVA). Corresponding with the decline in tone, \( R_Ca \) was significantly less in arteries from 24-mo-old rats compared with the other groups (Fig. 6, B and C).

**DISCUSSION**

The present study has shown that cerebral artery tone (endothelium intact) and intracellular \( Ca^{2+} \) ([Ca\(^{2+}\)]\(_i\)) sensitivity increased with age. These effects of aging on tone were, in part, caused by a change in
NOS-sensitive modulation. Another endothelium-derived substance, insensitive to NOS inhibition, declined with age. After removal of the endothelium, both tone and \([\text{Ca}^{2+}]_i\) were less in arteries from aged compared with adult rats.

**Aging and cerebral artery tone.** Tone is a sustained contractile state of cerebral arteries (6, 15, 16). In this study, arteries from all groups developed tone (endothelium intact), although the magnitude was significantly greater in arteries from aged (24 mo) compared with adult (6, 12, and 20 mo) rats. Few previous studies have examined the effects of age on cerebral artery responses to pressure. One reported that aging did not affect tone of isolated human pial vessels with diameters ranging from 300 to 1,200 \(\mu\)m (45). However, meaningful comparisons between human and rat studies are difficult because the range of vessel diameters in the human cerebral circulation is far greater than in the rat (10). Additionally, environmental factors and life span differences between species may affect responses to pressure (12).

Another aging study, conducted in vivo with pressurized cerebral microvessels (500–1,200 \(\mu\)m), found that artery diameters were similar between aged (24–27 mo) and adult (3–6 mo) Fischer rats (35). These negative aging results may have resulted from use of cerebral arterioles with diameters smaller than arteries used in the present study. Additionally, in vivo studies are more complex because a number of concurrent influences (intraluminal flow and perivascular nerve activity) modify development of tone (6, 21). Overall, the present study is the first to show that aging affects development of tone in resistance-sized, rat cerebral arteries.

**Aging and \([\text{Ca}^{2+}]_i\).** The \([\text{Ca}^{2+}]_i\) levels in arteries from aged rats (24 mo) were significantly greater than in the other groups at 20 and 40 mmHg (Fig. 2C) corresponding with greater tone in these arteries (Fig. 1E). Nu-

![Fig. 4. Effect of intraluminal pressure on \(R_{Ca}\) in endothelium-intact arteries treated with L-NAME from rats of different ages. \(R_{Ca}\) responses to increasing pressures in PSS (open bars) or treated with 100 \(\mu\)M L-NAME (solid bars) at 40 mmHg (A) and 80 mmHg (B) are shown. \(R_{Ca}\) was calculated as the percent difference at either 40 or 80 mmHg (PSS or L-NAME) from \(R_{Ca}\) at 10 mmHg (PSS). Values are means ± SE. *Significant differences (\(P < 0.05\)) from PSS as determined by ANOVA. †Significant differences (\(P < 0.05\)) from 6- (\(n = 11\)), 12- (\(n = 11\)), and 20-mo-old rat (\(n = 7\)) responses as determined by ANOVA.

![Fig. 5. Effects of intraluminal pressure on diameter of endothelium-denuded arteries from rats of different ages. Diameter responses to increasing pressures in arteries treated with L-NAME (solid symbols) and after endothelium disruption (−Endo; open symbols) from 6- (A; \(n = 11\)), 12- (B; \(n = 11\)), 20- (C; \(n = 7\)), and 24-mo-old rats (D; \(n = 6\)) are shown. *Effect of pressure on L-NAME-insensitive constrictions. L-NAME-insensitive constriction was determined by subtracting the diameters in L-NAME from diameters after endothelium disruption (−Endo). Values are means ± SE. *Significant difference (\(P < 0.05\)) from L-NAME as determined by ANOVA. †Significant difference (\(P < 0.05\)) from 6-, 12-, and 20-mo-old rats as determined by ANOVA.
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pressures is an age-dependent decline in the function of sarcoplasmic reticulum (SR) Ca\(^{2+}\)-ATPase pumps. Higher basal [Ca\(^{2+}\)]\(_i\) correspond with impaired Ca\(^{2+}\)-ATPase activity in aortas from aged compared with adult rats (31). Similarly, studies in sympathetic nerves in the rat tail artery and dorsal root ganglion cells from Fischer rats suggest that there is an age-related decline in SR-mediated [Ca\(^{2+}\)]\(_i\), buffering (24, 41). Measurement of vascular smooth muscle cell membrane potential and buffering capacity of the SR will be required to determine the age-dependent effects on [Ca\(^{2+}\)]\(_i\).

[Ca\(^{2+}\)]\(_i\) differences between groups were abolished at higher pressure (Fig. 2), indicating that arteries from 24-mo-old rats developed greater tone (Fig. 1) through a mechanism altering [Ca\(^{2+}\)]\(_i\), sensitivity. Our present findings contrast with data reported by Robert et al. (43). That study found that aging caused [Ca\(^{2+}\)]\(_i\) sensitivity to decline in response to norepinephrine using perfused rat tail arteries (43). The difference between our data and the data reported by Robert et al. may be a consequence of artery location and function. In future [Ca\(^{2+}\)]\(_i\) sensitivity studies, resistance-sized vessels must be investigated in the absence of second-messenger pathways (e.g., protein kinase C, Rho kinase) to precisely determine whether Ca\(^{2+}\) sensitivity changes with age (6).

Aging and NO. Endothelium-derived substances modulate tone of rat cerebral arteries (11, 16), yet little is known about these modulators in response to aging. Our data with L-NAME are the first to show that NO signaling was greater in cerebral arteries from 20-mo-old rats compared with 6- or 12-mo-old rats (Fig. 3). These data resemble findings by Muller-Delp et al. (37) in which contractile effects of L-NAME increased with age in skeletal muscle arterioles from Fisher 344 rats.

To interpret the effects of aging on NOS-sensitive signaling, we must also consider that another endothelium-derived substance, insensitive to L-NAME, modulated tone (Fig. 5). In arteries from 6- and 12-mo-old rats, NOS-sensitive and -insensitive mechanisms contribute equally to diameter regulation. At 20 mo of age, NOS-sensitive modulation appeared to compensate for an age-related decline in NOS-insensitive modulation. This was evident by a decline in L-NAME-insensitive constriction (Fig. 5) that was equal in magnitude to the upregulation of L-NAME-sensitive constriction (Fig. 3). Compensation between NOS-sensitive and -insensitive mechanisms has been reported in isolated, pressurized cerebral arteries (15). That study showed that treatment with indomethacin [cyclooxygenase (COX) inhibitor] upregulated NO-dependent signaling. Whether COX or another endothelium-derived factor is the NOS-insensitive substance is a goal of future experiments.

As rats aged from 20 to 24 mo, L-NAME-insensitive constrictions were nearly abolished (Fig. 5), indicating an age-dependent decline in NOS-insensitive signaling. Unexpectedly, L-NAME-sensitive constriction was similar to L-NAME effects in arteries from 6- and 12-mo-old rats (Fig. 3). We interpret these results as a
Evidence for an age-dependent decline in NO signaling requires additional interpretation of the data in Figs. 3 and 5. We believe that the full magnitude of NOS-sensitive modulation can only be determined after first inhibiting the NOS-insensitive mechanism. In other words, without NOS-insensitive mechanisms first being blocked, the data with L-NAME in Fig. 3 may underestimate the total L-NAME-sensitive constriction in arteries from 6-, 12-, and 20-mo-old rats. But, more importantly, underestimation of L-NAME-sensitive constriction in arteries from the 6, 12, and 20-mo-old rats would mask the decline in NOS signaling in arteries from 24-mo-old rats.

Many studies support an age-dependent decline in NO signaling (3, 33). However, an age-dependent change between compensating endothelium-derived substances is novel. Recently, compensation between endothelium-derived substances was shown to be age dependent in pressurized skeletal muscle arterioles (37). In that study, treatment with either indomethacin or L-NAME alone increased tone in arteries from aged but not adult rats. When indomethacin and L-NAME treatments were combined, tone increased equally in both age groups, supporting our hypothesis that aging affects compensation between NOS-sensitive and -insensitive substances.

An alternative explanation for the smaller contractile effects of L-NAME in arteries from 24-mo-old rats is that these vessels were closer to their contractile limits compared with arteries from the other groups. Support for this hypothesis is shown in Fig. 6A where tone (endothelium denuded) was significantly less in arteries from 24-mo-old rats compared with the other groups. These data indicate that the maximal contractile response to pressure declined with age, which may have indirectly limited L-NAME-sensitive constriction. Previous work in rat skeletal muscle arterioles supports our findings that tone declines in 24-mo-old compared with 4-mo-old Fischer 344 rats (36). Furthermore, both structural and mechanical characteristics of rat cerebral arterioles change during aging (14, 17). For example, contractile responses to receptor-mediated or depolarizing agents have been shown to decline (7, 17, 33) with age in rat cerebral and peripheral arteries. Measurement of responses to receptor-mediated and depolarizing substances will determine whether age affects the contractile capacity of cerebral arteries from Fischer 344 rats.

Aging, NOS signaling, and [Ca\(^{2+}\)]i regulation. NO modulates tone by decreasing smooth muscle [Ca\(^{2+}\)]i and/or Ca\(^{2+}\) sensitivity of the contractile filaments (2, 4, 8, 10). NOS inhibition caused a substantial [Ca\(^{2+}\)]i increase in arteries from 6-, 12-, and 20-mo-old rats without a significant age effect (Fig. 4). However, in arteries from aged rats (24 mo), NOS inhibition increased tone (Fig. 3) without any further change in [Ca\(^{2+}\)]i (Fig. 4). The effects of NO on [Ca\(^{2+}\)]i may depend on membrane potential repolarization through SR-dependent activation of K\(_{ca}\) channels and closure of L-type channels (22). In contrast, the mechanism of [Ca\(^{2+}\)]i-independent diameter regulation in arteries from 24-mo-old rats might decrease Ca\(^{2+}\) sensitivity of the contractile filaments (8) through alterations in activities of myosin light chain phosphatase (28), protein kinase C (28), heat shock protein 20 (23), or the small G protein RhoA (44).

Aging and NOS insensitivity. Although the identity of the NOS-insensitive substrate was not determined in the present study, aging is known to depress both endothelial-derived PGI2 (38) and EDHF (48). Hyperpolarization of smooth muscle cells by EDHF causes relaxation through closure of L-type channels (11). Thus, if age had depressed EDHF production or function, then [Ca\(^{2+}\)]i levels should have increased after removal of the endothelium compared with [Ca\(^{2+}\)]i levels in the presence of L-NAME (Fig. 6). This clearly did not occur.

Another possibility is that synthesis and/or release of PGI2 or a related COX-sensitive substance was depressed with age. In aortic endothelial cells, basal and thrombin-stimulated release of PGI2 was significantly less in old (25 mo) compared with young (1–2 mo) rats (38). Similar age-related declines in PGI2 release have been observed in human umbilical and bovine aortic endothelial cells (19). Presently unresolved is whether PGI2-induced dilation requires Ca\(^{2+}\)-dependent (29) or -independent (40) mechanism in pressurized cerebral arteries and whether these mechanisms are age sensitive.

Aging and cerebral autoregulation. What might be a consequence of greater artery tone to the cerebral circulation of aged rats? Previous aging studies demonstrated that the lower limits of CBF autoregulation are shifted toward higher pressures without an effect on resting CBF in aged rats (13, 26). An age-related increase in cerebral artery tone would not influence cerebral perfusion or vascular resistance in the resting animal. However, greater tone may diminish the ability of the cerebral circulation to redistribute CBF during cardiovascular stress. For example, an age-dependent upward shift in the lower limits of CBF autoregulation diminished dilatory responses to hypotension in basilar arteries from aged (24 mo) compared with adult (6 mo) rats (46). Therefore, the potentially deleterious effect of greater tone caused by advancing age may only become discernible at the limits of CBF autoregulation.

In summary, we have shown that aging increased cerebral artery tone (endothelium intact) and sensitivity to intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]j). Aging had additional effects on tone through both NOS-sensitive and -insensitive mechanisms. After removal of the endothelium, both tone and [Ca\(^{2+}\)]i were less in arteries from aged compared with adult rats. These changes in arterial reactivity to pressure may have consequences in regard to affecting autoregulation of cerebral blood flow in the aging rat.
DISCLOSURES

This work was supported in part by National Heart, Lung, and Blood Institute Grant R01-HL-69078-01 (to G. G. Geary) and American Heart Association Grant 0040021N (to J. N. Buchholz).

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