Aging Increases PGHS-2–Dependent Vasoconstriction in Rat Mesenteric Arteries

Ken G. Stewart, Yunlong Zhang, Sandra T. Davidge

Abstract—During aging, the vascular endothelium changes functionally and morphologically. Although previous studies have shown that endothelium-derived eicosanoids increase vessel tone in aging, the precise mechanism(s) has not been fully determined. We hypothesized that aging would increase prostaglandin H synthase (PGHS)-dependent vasoconstriction as well as decrease nitric oxide–dependent relaxation. Mesenteric arteries from 3-month-old (n=9) and 12-month-old (n=14) female Sprague-Dawley rats were studied in a myograph system. Aging significantly blunted the endothelium-dependent relaxation response to methacholine compared with young rats (EC_{50}=7.77\times10^{-8} \text{ mol/L}, P<0.05). Nitric oxide synthase inhibition reduced methacholine-induced relaxation in the young (P<0.05) but had no effect in the aging group. Specific inhibition of the PGHS-1 isoform did not significantly affect methacholine-mediated relaxation in the young or aged groups. However, PGHS-2 inhibition greatly enhanced relaxation to methacholine (1.59\times10^{-8} \text{ versus } 7.77\times10^{-8} \text{ mol/L}, P<0.01) in the aged group only, restoring vessel function to that of the young. In the aged group, inhibition of the prostaglandin H_{2}/thromboxane A_{2} receptor enhanced methacholine-dependent relaxation similar to that of PGHS-2 inhibition. Moreover, arterial expression of PGHS-2 protein increased with age. In summary, nitric oxide–dependent modulation of vessel function decreased with age, PGHS-1 did not significantly affect vessel tone in either the young or aging group, and PGHS-2 greatly increased vasoconstriction in aging. Thus, we have identified enhanced PGHS-2–mediated vasoconstriction in aging and therefore suggest that inhibition of this isoform is potentially a new target for therapeutic intervention to improve vascular function. (Hypertension. 2000;35:1242-1247.)

Key Words: prostaglandins ■ vasculature ■ aging ■ nitric oxide ■ endothelium

The process of aging is associated with a variety of deleterious adaptations that can have pathological effects on the vasculature. For example, during aging, the production of superoxide anions increases\(^1\)–\(^2\) whereas antioxidant systems are suppressed\(^3\)–\(^5\) and production of the vasodilator nitric oxide (NO) is decreased.\(^5\) Although decreased endothelium-dependent relaxation\(^6\),\(^7\) is associated with aging, the specific mechanisms for such alterations in vascular responsiveness are not completely understood.

The endothelium contributes to the regulation of vessel tone by releasing vasodilators and vasoconstrictors\(^8\) that modulate both physiological and pathophysiological processes. The nitric oxide synthase (NOS) and prostaglandin H synthase (PGHS) pathways are of particular importance because they have a substantial influence on vessel function and are affected by the processes of aging.\(^7\),\(^9\)–\(^12\) Indeed, there is an imbalance in which PGHS-dependent vasoconstriction becomes much more prominent with age and results in significantly greater vessel tone.\(^7\),\(^9\)–\(^12\) Reactive oxidative species\(^13\),\(^14\) and cytokines\(^15\),\(^16\) are both age-related factors capable of increasing expression of the inducible PGHS isoform (PGHS-2), through activation of the transcription factor nuclear factor-κB.\(^17\),\(^18\) Similarly, conditions of elevated oxidative stress favor the reaction between superoxide anions and NO, thereby consuming the vasodilator NO.\(^19\),\(^20\)

We have previously shown that aging increases the level of oxidative stress as well as enhances PGHS-dependent vasoconstriction.\(^12\) However, the differential modulation of vessel function by PGHS-1 versus PGHS-2 has not been determined. Given the age-associated increase in PGHS-2–inducing factors such as reactive oxidative species\(^1\),\(^2\) and cytokines\(^21\)–\(^23\) as well as predominance of PGHS-2 in conditions such as Alzheimer’s disease\(^24\) and arthritis,\(^25\) we hypothesized that the PGHS-2 isoform contributes to increased vessel tone in aging. Moreover, determining the effect of PGHS-2 on vascular function in aging could lead to the clinical use of newly developed, specific PGHS-2 inhibitors. Therefore, the goals of this study were to further characterize the changes in vascular function during aging; specifically, the modulation of vascular function by the NOS and PGHS pathways were determined.

Methods

General Animal Model
Female Sprague-Dawley rats (2 months of age) were obtained from Biological Sciences and housed in the facilities at the University of...
The rats were either 3 months (n=9) or 12 months (n=14) of age. On the day of the experiment, they were killed under light anesthesia with methohexital sodium (50 mg/kg body wt). The animal protocols were examined by the University of Alberta Animal Welfare Committee and found to be in compliance with the guidelines issued by the Canada Council on Animal Care.

Experimental Design
Mesenteric arteries averaging 250 μm in diameter were mounted in an isometric myograph system. Four separate baths were used to study arterial segments simultaneously. Cumulative doses of phenylephrine (1 to 50 μmol/L) were administered to measure sensitivity and determine the dose that would give a 50% constriction (EC50) for each individual artery. The EC50 of phenylephrine was used to constrict arteries to achieve a baseline from which subsequent relaxation responses were measured. After completion of each dose-response curve, a 30-minute recovery period was allowed, during which the baths were changed every 10 minutes with fresh HEPES-buffered physiological saline solution (sodium chloride 142, potassium chloride 4.7, magnesium sulfate 1.17, calcium chloride 1.56, potassium phosphate 1.18, HEPES 10, and glucose 5.5 mmol/L, pH 7.4). The studies performed involving the absence and presence of inhibitors were conducted sequentially on the same artery. Vessels were preincubated with inhibitors for 15 minutes before methacholine dose-response curves (1 mmol/L to 1 μmol/L).

Methacholine relaxation curves were generated in the absence or presence of the NOS inhibitor N\(^6\)-monomethyl-L-arginine (L-NMMA, 250 μmol/L) to determine a role for NO in the responses. To verify the pharmacological inhibition of the NOS pathway, dose-response curves to L-NMMA (3 μmol/L to 30 μmol/L) were performed in arteries preconstricted to 10% of their maximum response to phenylephrine. Sequential doses of L-NMMA were then applied to the vessels, and the level of constriction in response to each dose of inhibitor was recorded. Maximum vessel constriction was reached at concentrations of L-NMMA below that used experimentally, suggesting that NOS activity was optimally inhibited. As well, the effect of the PGHS inhibitor meclofenamate (10 μmol/L), PGHS-1 inhibitor valeryl salicylate (3 mmol/L), PGHS-2 inhibitor NS-398 (10 μmol/L), and thromboxane A\(_2\) (TXA\(_2\))/prostaglandin H\(_2\) (PGH\(_2\)) receptor antagonist SQ-29548 (1 μmol/L) on methacholine-induced relaxation were measured.

Vascular smooth muscle sensitivity to the exogenous NO donor sodium nitroprusside (SNP) (1 nmol/L to 1 μmol/L), TXA\(_2\) mimetic U-46619 (1 mmol/L to 0.1 μmol/L), and phenylephrine were also measured. For these dose-response curves, arteries from the same rats were denuded of endothelium. Endothelium removal was done mechanically by threading a human hair through the lumen of the artery. Confirmation of complete endothelium removal was assessed pharmacologically with a single dose of 1 μmol/L methacholine.

The reproducibility of repeating curves for these experiments was determined in a preliminary set of experiments designed to test for tachyphylaxis. As previously described, Western immunoblotting was performed for PGHS-1 and PGHS-2 with primary monoclonal antibodies (mouse anti–PGHS-1 and anti–PGHS-2; Cayman Chemical Co).

Results
There was no significant difference in response to phenylephrine (endothelium intact or denuded) between the 2 groups. Intact mesenteric arteries preconstricted with their EC50 of phenylephrine demonstrated a concentration-dependent relaxation response to methacholine in the young and aged groups. Although both groups relaxed to 100%, the aged group had a substantially blunted relaxation response to methacholine, as indicated by a larger EC50 value (Figure 1). PGHS inhibitors were used to determine the role of the prostaglandin pathway with respect to the altered vascular responses in the aged group. The PGHS inhibitor meclofenamate significantly enhanced relaxation of intact vessels in the aged group but had no effect on the young (Figure 2A). In the presence of meclofenamate, there was no difference between the 2 groups. To determine which isoform was predominantly responsible for the augmented vasoconstriction in the aged group, specific PGHS-1 and PGHS-2 inhibitors were used. Valeryl salicylate, a PGHS-1 inhibitor, did not affect vessel relaxation in either group (Figure 2B). Conversely, the PGHS-2 inhibitor NS-398 greatly enhanced relaxation in the aged group and restored vessel function to that of the young group, which was not influenced by the inhibitor (Figure 2C). To elucidate the identity of the vasoconstrictive eicosanoid(s) in the aged group, the TXA2/PGHS receptor antagonist SQ-29548 was applied to the baths. Similar to PGHS inhibition, the receptor blocker did not affect relaxation in the young group (Figure 2D). However, inhibiting the receptor in the aged group enhanced relaxation in a manner similar to PGHS-2 inhibition (Figure 2D).

The role of NO in methacholine-induced relaxation in intact vessels was determined by inhibiting the NOS pathway with L-NMMA. Inhibiting NO production in the young group significantly blunted the relaxation response but did not prevent vessels from reaching maximal relaxation (Figure 3).
Figure 2. Bar graphs showing EC$_{50}$ values for methacholine alone (solid bars) and methacholine in presence of PGHS pathway inhibitors (open bars) in intact mesenteric arteries from young (3 months) and aged (12 months) rats. A, Meclofenamate: Inhibitor of both PGHS isoforms (young: n = 9; aged: n = 8); B, valeryl salicylate: PGHS-1 inhibitor (young: n = 9; aged: n = 8); C, NS-398: PGHS-2 inhibitor (young: n = 7; aged: n = 8); and D, SQ-29548: TXA$_2$/PGH$_2$ receptor blocker (young: n = 6; aged: n = 8). Bars represent mean±SEM.

*P < 0.05 vs methacholine alone, #P < 0.05 vs young.
In contrast, NOS inhibition did not affect relaxation to methacholine in the aged group (Figure 3), suggesting that NO was not mediating this relaxation response.

Smooth muscle sensitivity was assessed in endothelium-denuded vessels. Complete removal of the endothelium was confirmed by the absence of vessel response to methacholine. A TXA2 receptor agonist and exogenous NO donor were applied to endothelium-denuded vessels to determine whether or not smooth muscle sensitivity was partially responsible for the differential responses of vessels in the 2 groups. There was no difference in sensitivity or maximum responses between the young and aged vessels when a dose-response curve to the thromboxane mimetic U-46619 was measured (Figure 4A). As well, endothelium-denuded vessels in the 2 groups responded similarly to SNP (Figure 4B).

To provide insight into the cellular mechanisms determining vessel function, protein expression was measured in mesenteric arteries. As expected on the basis of the functional data, there was no significant difference in PGHS-1 protein expression in the young and aged group (arbitrary units = 339 ± 74 versus 429 ± 14). In agreement with the functional results, PGHS-2 protein expression in the mesenteric arteries was significantly higher in the aged group compared with the young group (Figure 5).

**Discussion**

The purpose of this study was to further characterize the changes in vascular function that occur during aging. Although we previously demonstrated that PGHS-dependent vasoconstriction is increased in rat mesenteric vessels during aging, it has not previously been determined whether PGHS-1 or PGHS-2 is the primary isoform responsible for the increase in vasoconstriction. In the present study, we found that PGHS-2 greatly contributes to vasoconstriction in mesenteric arteries during aging, whereas PGHS-1 does not have a significant effect on methacholine-induced relaxation in the young or aged group. Collectively inhibiting both PGHS isoforms or selectively inhibiting PGHS-2 resulted in the aged vessels functioning similar to young vessels under control conditions (without an inhibitor). Furthermore, blocking the TXA2/PGH2 receptor also eliminated the blunted relaxation in the aged vessels. Thus, modulation of vessel function through the TXA2/PGH2 receptor becomes much more dominant in aging as a result of increased PGHS-2 activity. This is very significant with the advent of specific PGHS-2 inhibitors that could potentially reverse the blunted relaxation associated with aging.

In contrast, NOS inhibition did not affect relaxation to methacholine in the aged group (Figure 3), suggesting that NO was not mediating this relaxation response.
Functional differences between young and aged vessels could be a consequence of both endothelial cell product formation and vascular smooth muscle sensitivity. However, our data suggest that the functional changes were the result of altered endothelial metabolism rather than changes in smooth muscle responsiveness. There was no difference in smooth muscle sensitivity to exogenous NO, because SNP induced similar relaxation in both groups. Moreover, the thromboxane mimetic U-46619 induced vessel constriction to a similar degree in young and aged vessels. Therefore, the enhanced vessel tone associated with the aged group is not due to increased PGH$_1$/TXA$_2$ receptor responsiveness.

The present study clearly demonstrates a vasoconstrictive influence of PGHS-2 products on vascular function during aging. Similar to the data of Dewitt et al. $^{28}$ who found that the concentration of PGHS is $\sim$20 times greater in the aortic endothelium than in the smooth muscle, our data suggest that the endothelium is the primary source of PGHS-2. Young and aged vessels responded similarly to the smooth muscle agonist phenylephrine. As well, methacholine did not affect vessel tone in endothelium-denuded vessels. Thus, it appears that stimulation of the endothelium is necessary to induce the age-associated increase in vessel tone. In agreement with the functional work, our preliminary immunohistochemistry data on aortic rings from the same animals indicated that PGHS-2 protein expression, localized in the endothelium, significantly increased with age.

Western immunoblot data suggest that increased PGHS-2 protein mass in mesenteric arteries contributes to the enhanced vasoconstriction in aging, whereas PGHS-1 protein mass did not change with age. There is a variety of potential mechanisms for the augmented role of PGHS-2–dependent vasoconstriction. For instance, both cytokines and oxidative stress become more prominent with age$^{21-23}$ and are capable of increasing PGHS-2 expression.$^{15,16,29}$ Therefore, further studies delineating these factors are necessary.

As well, we found that the role of NO in methacholine-induced relaxation decreases with age. When the NOS inhibitor L-NMMA was added to the vessel baths, relaxation was significantly blunted in the young group; suggesting that NO plays a dominant role in relaxing these vessels. Conversely, NOS inhibition did not affect relaxation to methacholine in the aged group. Thus, other factors appear to be responsible for relaxing small arteries in aging. Endothelium-derived hyperpolarizing factor has been shown to be a dominant regulator of relaxation in microvessels.$^{30,31}$ However, past studies have reported that endothelium-derived hyperpolarizing factor is more prominent in young than in aged rats.$^{32}$ Nonetheless, our observation of decreased NO-mediated relaxation in mesenteric arteries during aging is in agreement with Cernadas et al.$^{10}$ who suggest that eNOS-mediated relaxation decreases with age in the aorta.

In summary, NO-dependent modulation of vessel function decreased with age, PGHS-1 did not significantly affect vessel tone in either the young or aged group, and PGHS-2 greatly increased vasoconstriction in aging. Thus, we have identified enhanced PGHS-2–mediated vasoconstriction in aging and therefore suggest that inhibition of this isoform is potentially a new target for therapeutic intervention to improve vascular function.

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References


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