Effect of behavioral stress on coronary artery relaxation altered with aging in BHR

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Giulumian, Ararat D., Shawn G. Clark, and Leslie C. Fuchs. Effect of behavioral stress on coronary artery relaxation altered with aging in BHR. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R435–R440, 1999.—Behavioral stress and aging are associated with an increase in vascular disease. This study determined the mechanisms contributing to changes in endothelium-dependent relaxation of isolated coronary arteries (300–350 µm) induced by exposure to 10 days of air-jet stress (2 h/day) in young (3 mo) and old (18 mo) male borderline hypertensive rats (BHR). Aging, alone, did not alter endothelium-dependent relaxation to acetylcholine (ACh) quantitatively but did alter the mechanisms contributing to relaxation to ACh, which was largely dependent on nitric oxide synthase (NOS) in vessels from old, but not young, BHR. Behavioral stress resulted in an enhanced relaxation to ACh that was dependent on NOS in vessels from young stressed compared with young control BHR. Conversely, relaxation to ACh was reduced in coronary arteries from old stressed compared with old control BHR. In vessels from old control BHR, there was an NOS-independent component of relaxation mediated by opening of K+ channels that was absent in vessels from old stressed BHR. The superoxide anion scavenger, tiron, partially restored relaxation, and inhibition of cyclooxygenase largely restored relaxation to ACh in vessels from old stressed BHR. In summary, the effect of behavioral stress was age dependent. ACh-induced relaxation of coronary arteries was enhanced in an NOS-dependent manner in young BHR and was impaired in old BHR due to superoxide anions, vasoconstrictor cyclooxygenase products, and a loss of K+ channel-mediated relaxation.

endothelium-derived relaxing factors; nitric oxide synthase; cyclooxygenase

Behavioral stress is associated with the development of atherosclerosis, hypertension, cardiac arrhythmias, and sudden death (18, 21, 33–35). A recent clinical investigation found that exaggerated blood pressure responses during mental stress were associated with enhanced carotid atherosclerosis (17). Behavioral stress alters endothelium-dependent vascular relaxation in coronary arteries and in other vascular beds (13, 14, 33, 36, 39). These stress-induced alterations in vascular relaxation could contribute to the development of pathological conditions.

Aging is associated with an increased frequency of cardiovascular disease, even in the absence of other cardiovascular risk factors (25). The incidence of hypertension, heart failure, or ischemic heart disease is higher in aged than in young individuals (11). Studies have suggested that the higher incidence of cardiovascular disease in aged individuals may be attributed to enhanced cardiovascular responsiveness to environmental stressors (28, 31). An increase in sympathetic nervous system activity and plasma catecholamines occurs with aging (9, 12). Additionally, vascular responsiveness to both vasodilatory and vasoconstrictor substances is altered, and vascular compliance is decreased with aging (23, 27, 37, 38).

The borderline hypertensive rat (BHR), a first-generation offspring of a female spontaneously hypertensive rat (SHR) and male Wistar-Kyoto (WKY) rat, has been used extensively for studies on behavioral stress due to its sensitivity to several environmental challenges (33). BHR exhibit a resting mean arterial pressure of ~130 mmHg and do not develop the age-related increase in arterial pressure observed in SHR (33). Some studies indicate that BHR have exaggerated cardiovascular and sympathoadrenal responses to acute stress compared with normotensive controls (16, 33). However, this remains controversial (19). Prolonged exposure of BHR to air-jet stress results in sustained hypertension that persists after removal of the stress. However, changes in endothelium-dependent vascular relaxation occur before the development of stress-induced sustained hypertension in adult (13–14 wk old) BHR (13, 14). The effect of behavioral stress on endothelium-dependent vascular relaxation has not been evaluated in aged BHR. Because both behavioral stress and aging can alter vascular reactivity, and aging has been reported to enhance sensitivity to behavioral stress, the present study determined the effects of behavioral stress on endothelium-dependent relaxation of coronary arteries from young (13–14 wk old) and old (72–74 wk old) BHR. Acetylcholine (ACh), which produces endothelium-dependent relaxation and releases several vasodilatory substances, including NO, prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF), was used to elicit endothelium-dependent relaxation in these studies (10, 26).

Methods

BHR. Female SHR and male WKY rats were obtained from Taconic Farms and were bred at the Medical College of Georgia to obtain the first-generation offspring BHR. Male BHR (13–14 wk or 72–74 wk) were randomly divided into two groups (control or stressed). The stressed group was exposed to air-jet stress for 2 h/day for 10 days, whereas the control group remained in its home cage for 10 days.

Air-jet stress. Rats were positioned in tubular Plexiglas restrainers and were placed in sound-insulated chambers. Chronic stress consisted of pulses of compressed air (15 lb/in.2).
directed toward the face from a ¼-in. opening at the front of the restrainer. Animals were subjected to a random duration of pulses (5–120 s) and interpulse intervals (5–120 s) for 2 h/day for 10 days.

Hemodynamic measurements. After the exposure to air-jet stress on day 10, rats were anesthetized with ketamine (50 mg/kg im) and acepromazine (16 mg/kg im). Under aseptic conditions, a cannula (PE-50 attached to PE-10) was placed in the femoral artery and was exteriorized at the nape of the neck. The cannula was flushed with heparinized saline (100 U/ml). After a 1-day recovery period, arterial pressure and heart rate were monitored with a Grass recorder in unrestrained rats in their home cage. Heart rate was derived with a cardiotachometer that was triggered from the arterial pressure pulse.

Coronary artery endothelium-dependent relaxation. After hemodynamic values were obtained, rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and heparin (500 units) was administered in the left ventricle. Hearts were removed and placed in modified Krebs-Ringer bicarbonate solution (composition in mM: 118.3 NaCl, 4.7 KCl, 2.5 NaHCO3, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, and 11.1 dextrose) that had been chilled and oxygenated (20% O2 and 5% CO2). A left ventricular coronary artery (300–350 µm diameter and 1–2 mm long) was isolated from surrounding cardiac tissue microscopically.

The artery was transferred to a vessel chamber and was mounted between two glass micropipettes (100-µm-diameter tips) with 10–0 ophthalmic suture. The tissue bath was transferred to the stage of an Olympus inverted light microscope coupled to a monitor and video dimension analyzer (Living Systems Instrumentation, Burlington, VT). Coronary artery intraluminal diameter was monitored continuously on a Grass recorder.

Oxygenated (20% O2, 5% CO2) Krebs-Ringer solution was maintained at 37°C and was continuously circulated through the tissue bath. The lumen of the vessel was filled with Krebs-Ringer solution through the micropipette and was maintained at a constant pressure of 40 mmHg. The vessel was allowed to equilibrate for 1 h. In vessels preconstricted to 30–50% of baseline diameter with endothelin-1 (ET-1), dose-response curves to ACh (10–4 to 3 × 10–5 M) were performed in the absence and presence of inhibitors, including N-nitro-L-arginine (L-NNA, 1 mM), an inhibitor of nitric oxide synthase (NOS) activity, indomethacin (Indo, 10 µM), an inhibitor of cyclooxygenase, or tiron (10 mM), a superoxide anion scavenger. All inhibitors were added to the vessel bath 20 min before performing the dose-response curve to ACh. To determine the role of K+ channels in relaxation to ACh, a dose-response curve to ACh was performed in vessels preconstricted to 30–50% of baseline diameter with KCl (25–50 mM). This concentration range of KCl effectively blocks K+ efflux and prevents relaxation mediated by opening of K+ channels (5, 6). Finally, a dose-response curve to nitroprusside (NP; 10–8 to 3 × 10–4 M), an exogenous donor of NO, was performed in vessels preconstricted with ET-1. When possible, more than one coronary artery of similar size was obtained from the same rat, but each experiment was performed only one time per rat. Additionally, only one dose-response curve was performed per vessel.

Chemicals. All chemicals used in this study were obtained from Sigma Chemicals. ET-1 (human, porcine) was dissolved in 1% albumin (bovine) and was diluted with Krebs solution. All other agents were dissolved in nanopure water and were diluted in Krebs solution.

Data analysis. Data obtained from coronary vessels were expressed as intraluminal diameter in micrometers. Responses to vasodilatory agents were expressed as percent relaxation after preconstriction with ET-1. All data were reported as means ± SE. Statistical differences were determined by analysis of variance for repeated measures followed by Student’s modified t-test with Bonferroni correction for multiple comparisons. The criterion for significance was P < 0.05.

RESULTS

Age, hemodynamic measurements, and baseline coronary artery intraluminal diameter (at a constant intraluminal pressure of 40 mmHg, in vitro) from control and stressed BHR are shown in Table 1. Mean arterial pressure and heart rate in conscious, unrestrained rats were not significantly different in control compared with stressed rats or in young compared with old rats. Coronary artery intraluminal diameter was similar between groups.

Vascular reactivity. Coronary arteries were preconstricted to 47 ± 3, 48 ± 3, 46 ± 3, and 43 ± 6% of baseline diameter with ET-1 in young control, young stressed, old control, and old stressed groups, respectively. There were no significant differences in the percent preconstriction produced by ET-1 among groups for all dose-response curves. In the absence of addition of vasodilatory agents, the preconstriction produced by ET-1 was maintained at a constant level for at least 1 h, and, in the following experiments, all dose-response curves were completed within 30 min. A summary of the response to ACh after preconstriction with ET-1 is shown in Fig. 1. Exposure to behavioral stress inhibited endothelium-dependent relaxation to ACh in coronary arteries from old BHR and increased relaxation to ACh at doses of 3 × 10–6 and 10–5 M in vessels from young BHR. Aging, alone, did not alter relaxation ACh. However, after behavioral stress, there was a marked difference in endothelium-dependent relaxation in coronary arteries of young compared with old BHR. An exogenous nitric oxide (NO) donor, NP, produced similar, dose-dependent relaxation of coronary arteries from all four groups (Fig. 2).

The effect of inhibition of NOS activity with L-NNA on relaxation to ACh in coronary arteries from young control and stressed BHR is shown in Fig. 3A. L-NNA significantly inhibited relaxation to ACh in vessels from young stressed, but not from young control, BHR. As a result, relaxation to ACh in the presence of L-NNA was similar between groups. The effect of L-NNA on

| Table 1. Age, hemodynamic measurements, and coronary intraluminal diameter in BHR |
|---------------------------------------------|---|---|---|---|
| Age, days | OC | OS | YC | YS |
| Mean arterial pressure, mmHg | 120 ± 3 | 118 ± 5 | 126 ± 2 | 127 ± 2 |
| Heart rate, beats/min | 320 ± 8 | 313 ± 14 | 335 ± 4 | 340 ± 6 |
| Coronary intraluminal diameter, µm | 350 ± 17 | 355 ± 15 | 306 ± 17 | 327 ± 11 |

Values represent means ± SE. OC, old control; OS, old stressed; YC, young control; YS, young stressed. *P < 0.05 vs. respective old group.
relaxation to ACh in coronary arteries from old control and stressed BHR is shown in Fig. 3B. In vessels from old control BHR, L-NNA reduced relaxation to ACh (10^{-7} to 3 \times 10^{-6} M), but ACh still produced a maximum relaxation of 37 \pm 8\% in the presence of L-NNA. Conversely, L-NNA completely abolished relaxation to ACh in vessels from old stressed BHR. There was a significant difference between coronary arteries from old control and old stressed groups in the presence of L-NNA.

Because relaxation to ACh remained in the presence of L-NNA in coronary arteries from young control, young stressed, and old control BHR, the role of K^+ channels in this NOS-independent relaxation to ACh was determined. A dose-response curve to ACh was performed in coronary arteries pretreated with L-NNA and preconstricted with KCl. The preconstriction produced by KCl was 45 \pm 2, 42 \pm 2, and 42 \pm 4\% in vessels from young control (n = 5 rats), young stressed (n = 6 rats), and old control (n = 4 rats) BHR, respectively. Under these conditions, relaxation to ACh was completely abolished in all groups, suggesting that relaxation to ACh that remained after inhibition of NOS was due to opening of K^+ channels (data not shown).

To evaluate the role of superoxide anions in impairing relaxation to ACh in coronary arteries from aged BHR, vessels were pretreated with the superoxide anion scavenger tiron. A summary of the dose-response curve to ACh in the absence and presence of tiron is shown in Fig. 4. Tiron significantly enhanced relaxation to ACh (10^{-6} and 3 \times 10^{-6} M) in coronary arteries...
from old stressed BHR. However, relaxation to ACh was not completely restored. Tiron had no effect on relaxation to ACh in coronary arteries from old control BHR.

The role of cyclooxygenase products in the response to ACh in coronary arteries from old BHR was also determined. A summary of the results is shown in Fig. 5. Pretreatment of vessels with Indo decreased relaxation to ACh in vessels from old control BHR and increased relaxation to ACh in vessels from old stressed BHR. In the presence of Indo, relaxation to ACh was similar between old control and old stressed groups.

**DISCUSSION**

BHR have been used extensively for studies on behavioral stress because they are sensitive to several environmental challenges (33). Prolonged exposure of BHR to air-jet stress results in sustained hypertension, which persists after removal of stress (33). However, as observed previously (13, 14), exposure to only 10 days of air-jet stress did not alter the resting arterial pressure of conscious BHR in this study, allowing for the determination of behavioral stress-induced changes in coronary vascular endothelium-dependent relaxation before the development of hypertension. As observed by others in WKY rats and SHR (38), aging did not alter resting arterial pressure in BHR in this study.

Endothelial cells produce several relaxing factors, including NO, EDHF, and prostacyclin. These relaxing factors can be released after activation of muscarinic receptors with ACh, which is also capable of releasing endothelium-derived contracting factors such as thromboxane A2 and prostaglandins H2 and F2α (29). Endothelial NOS converts L-arginine to NO, which may produce relaxation by decreasing smooth muscle cell Ca2+ levels through a cGMP-dependent pathway or through hyperpolarization due to increased conductance of K+ channels (5, 15, 30). EDHF may be arachidonic acid metabolites of the cytochrome P-450 pathway, such as epoxyeicosatrienoic acids (2). Prostacyclin is produced by the action of prostacyclin synthase on endoperoxides, which are produced by cyclooxygenase.

Previously, we demonstrated that endothelium-dependent vascular relaxation is altered by exposure to behavioral stress in adult BHR (13, 14). The present study demonstrates that endothelium-dependent relaxation of coronary arteries was altered differentially by stress in young compared with old BHR. Others have reported differential effects of behavioral stress with aging in other animal models such as cardiomyopathic hamsters and in humans (3, 9). Aging, alone, did not alter relaxation to ACh quantitatively but did alter the mechanisms mediating relaxation to ACh. Endothelium-dependent relaxation to ACh was not altered by aging in coronary arteries of WKY rats or SHR (38). However, aging has been reported to alter vascular resistance and reactivity to exogenous and endogenous vasoactive agents in many vascular beds (1, 22, 27, 38, 41).

In coronary arteries from young BHR exposed to air-jet stress, NOS-dependent relaxation was increased. Unlike the effect of behavioral stress on young BHR, exposure to stress impaired endothelium-dependent coronary artery relaxation in old BHR. Exposure to behavioral stress also altered the mechanism mediating endothelium-dependent relaxation. Relaxation to ACh was completely dependent on NOS in coronary arteries from old stressed BHR. However, an EDHF contributed to ACh-induced relaxation of coronary arteries from young control, young stressed, and old control BHR, since relaxation that was resistant to inhibition of NOS was prevented by a high level of extracellular K+ in these groups. Because endothelium-dependent relaxation was completely dependent on NOS in vessels from old stressed BHR, behavioral stress may reduce relaxation mediated by EDHF in aged animals.

The cause of the differential effects of behavioral stress on old compared with young BHR is not known. One possibility is that there is an alteration in the susceptibility to stress-induced vascular changes in young compared with aged BHR. Another possibility may be related to the finding that the mechanisms contributing to ACh-induced relaxation are different in coronary arteries from old vs. young BHR in the absence of stress. Relaxation to ACh was largely dependent on NOS in vessels from old BHR, whereas NOS did not significantly contribute to relaxation in vessels from young BHR. Similar results were observed in mesenteric arteries of WKY rats in which an inhibitor of NOS activity had little effect on ACh-induced relaxation in vessels from young WKY rats but inhibited relaxation in vessels from old WKY rats (27).

Other studies on changes in NOS activity associated with aging are controversial. In one study, inhibition of NOS activity augmented contraction to norepinephrine in mesenteric resistance arteries of aged, but not adult, WKY rats, suggesting an enhanced activity of NOS associated with aging (22). However, in aorta of aged WKY rats, basal release of NO and expression of endothelial NOS mRNA were reduced (1). Endothelium-independent relaxation to the nitrovasodilator NP did not differ with age or stress. This demonstrates that the vascular responsiveness to exogenous NO remained...
unaffected and would not contribute to differences observed. It was previously reported that coronary artery endothelium-independent relaxation to NP was not altered by aging in WKY rats or SHR (38).

Relaxation to ACh that was resistant to inhibition of NOS was mediated by opening of K+ channels in coronary arteries from young and old control BHR. Because the K+ channel-mediated component of the response was reduced in vessels from old BHR, our findings would suggest that this mechanism of relaxation may be reduced with aging. This is supported by the finding that ATP-sensitive K+ channel-induced vasodilation of brain microvessels was impaired in aged compared with adult Sprague-Dawley rats (37).

In the present study, superoxide anions, which can inactivate NO, were found to contribute to impaired endothelium-dependent relaxation of coronary arteries from old stressed BHR. Conversely, scavenging of superoxide anions had no effect on relaxation to ACh in coronary arteries from old control BHR. With aging, there is a rise in plasma catecholamines and free radical-induced damage of cells (12). In aged Wistar rats, plasma superoxide dismutase activity was decreased (1). Because aging is associated with enhanced sympathetic nervous system activity and, in some studies, an enhanced responsiveness to behavioral stress, it can be speculated that aged BHR were more sensitive to stress and that enhanced levels of catecholamines may have led to higher levels of free radicals due to autooxidation of catecholamines (8, 9).

Although superoxide anions appear to contribute to the stress-induced decrease in endothelium-dependent relaxation, scavenging of superoxide anions did not completely restore relaxation to ACh. The results of this study indicate that behavioral stress may enhance production of, or sensitivity to, vasoconstrictor prostaglandins. Inhibition of cyclooxygenase had opposite effects on coronary arteries from aged control and stressed BHR. In coronary arteries from control BHR, vasodilatory prostanooids contributed to relaxation to ACh. Conversely, in coronary arteries from stressed BHR, vasoconstrictor prostanooids were released by ACh, masking the relaxation to ACh. Although the mechanism mediating this effect of behavioral stress is unknown, it is interesting to note that aging was associated with increased formation of the vasoconstrictor prostaglandin H2 (20).

In summary, the effect of behavioral stress on coronary artery endothelium-dependent relaxation is altered with aging in BHR. Exposure to stress produced an NOS-dependent increase in relaxation to ACh in vessels from young BHR and a decrease in relaxation to ACh in vessels from old BHR. The impaired response to ACh observed in old BHR was associated with superoxide anions, vasoconstrictor prostaglandins, and a loss of the component of relaxation that was L-NNA resistant and K+ channel mediated. The differential effect of stress may be related to the finding that mechanisms mediating relaxation to ACh are altered by aging in BHR.

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