β-Adrenergic receptor blockade impairs coronary exercise hyperemia in young men but not older men

Amanda J. Ross, Zhaohui Gao, Jonathan P. Pollock, Urs A. Leuenberger, Lawrence I. Sinoway, and Matthew D. Muller

Pennsylvania State University College of Medicine, Penn State Hershey Heart and Vascular Institute, Hershey, Pennsylvania

Submitted 20 August 2014; accepted in final form 15 September 2014

Ross AJ, Gao Z, Pollock JP, Leuenberger UA, Sinoway LI, Muller MD. β-Adrenergic receptor blockade impairs coronary exercise hyperemia in young men but not older men. Am J Physiol Heart Circ Physiol 307: H1497–H1503, 2014. First published September 19, 2014; doi:10.1152/ajpheart.00584.2014.—Patients with coronary artery disease have attenuated coronary vasodilator responses to physiological stress, which is partially attributed to a β-adrenergic receptor (β-AR)-mediated mechanisms. Whether β-ARs contribute to impaired coronary vasodilation seen with healthy aging is unknown. The purpose of this study was to investigate the role of β-ARs in coronary exercise hyperemia in healthy humans. Six young men (26 ± 1 yr) and seven older men (67 ± 4 yr) performed isometric handgrip exercise at 30% maximal voluntary contraction for 2 min after receiving intravenous propranolol, a β-AR antagonist, and no treatment. Isoproterenol, a β-AR agonist, was infused to confirm the β-AR blockade. Blood pressure and heart rate were monitored continuously, and coronary blood flow velocity (CBV) was evaluated with and without propranolol, a nonselective β-AR antagonist, and without propranolol, a nonselective β-AR agonist, to verify the β-AR blockade. Our hypothesis was that propranolol would impair CBV responses to isometric exercise in young and older men.

METHODS

Ethical approval. All study protocols were approved in advance by the Institutional Review Board of the Penn State Milton S. Hershey Medical Center and conformed to the Declaration of Helsinki. All subjects provided written and informed consent.

Subjects and design. This study employed a repeated-measures crossover design, whereby physiological parameters were continuously measured during baseline, pharmacological interventions, and isometric handgrip exercise. Age (young, older) served as a between-subjects factor and drug (control, propranolol) and time (baseline, exercise) as within-subjects factors. Six young men (26 ± 1 yr) and seven older men (67 ± 4 yr) volunteered to participate. The sample size was determined after the first four subjects in each group had completed testing. Specifically, we determined that, if the mean change in CBV between groups was 7 cm/s with a standard deviation of 4 cm/s, then we would need to enroll five subjects per group to be able to reject the null hypothesis with a probability of 0.80 and a type I error of 0.05. Because of expected person-to-person variability, we enrolled six young men and seven older men before conducting the formal statistical analysis. Women were excluded because of sex differences in β-AR control of coronary blood flow (28, 36).

All subjects had supine resting blood pressure (BP) below 125/80 mmHg and were nonasthmatic, nonobese, nonsmokers, and not taking any prescription or vasoactive medications. All subjects were in good health as determined by history and physical examination and reported being physically active, but none were competitive athletes. Before enrollment, all older subjects had a negative EKG monitored-exercise stress test (Bruce protocol) to exclude hemodynamically significant coronary artery disease. Subjects refrained from caffeine, alcohol, and exercise for 24 h before the study and arrived at the laboratory following an overnight fast.

Physiological and perceptual measurements. All study protocols were conducted in the supine or left lateral position in a dimly lit
thermoneutral laboratory (22–25°C) during the morning hours (7:30 AM to 12:00 PM). Upon arrival at the laboratory, subjects dressed in a high-density tube-lined suit (Med-Eng Systems) that covered the entire body except for the feet, hands, and head. Neutral water (34–35°C) was perfused through the suit to maintain mean skin temperature at a constant level because β-AR stimulation can affect thermogenesis (1, 4, 42, 57). Subjects were instrumented with a three-lead EKG (Cardio-cap/S; GE Healthcare) to monitor heart rate (HR), a finger BP cuff (Finometer, FMS), a pneumotrace to monitor respiratory movement, and an intravenous catheter in each arm. Before infusions and exercise, resting BPs were obtained in triplicate by automated oscillometry of the left brachial artery (Philips Sure Signs VS3) after 15 min of quiet rest and were used to verify the Finometer values as previously described (45). All of the variables listed above were collected at 200 Hz by PowerLab (ADInstruments). Basic pulmonary function was determined with a MiniSpir device (Medical International Research) in the left lateral position before and after propranolol. The variables of interest included forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC).

Peak diastolic CBV in the left anterior descending artery (LAD) was used an index of myocardial O2 supply and was obtained from the adjusted apical four-chamber view using a GE Vivid 7 echocardiography system (all images acquired by Z. Gao). The specific procedures for measuring CBV in the LAD have been previously described by our laboratory, and the reproducibility within subjects has been verified (18, 20–22, 40). Maximal voluntary contraction (MVC) of the hand and forearm was obtained using a handgrip dynamometer. Thirty percent of the MVC was calculated and used in subsequent trials; this workload performed for 2 min is known to raise sympathetic nerve activity (38) and rate pressure product (RPP) (i.e., needed for measuring CBV in the LAD have been previously described by our laboratory, and the reproducibility within subjects has been verified (18, 20–22, 40). Maximal voluntary contraction (MVC) of the hand and forearm was obtained using a handgrip dynamometer.

Study protocol. Figure 1 displays the experimental timeline. Following baseline measurements in the supine posture, isoproterenol hydrochloride (Marathon Pharmaceuticals; 1 mg in 500 ml saline) was infused through an intravenous catheter in the left arm at 0.5 μg/min (or 15 ml/h) for 1 min, and this infusion rate increased by 0.5 μg/min each minute until HR increased by ≥25 beats/min. After a 30-min washout period, a loading dose of propranolol hydrochloride (Hikma Pharmaceuticals) was intravenously infused in the right arm over 15 min (0.25 mg/kg at a rate of 4 ml/min) followed by a maintenance infusion (0.006 mg/kg per min at a rate of 1.45 ml/min) for the remainder of the study, similar to other paradigms (8, 49). Pulmonary function was measured in the left lateral position before the start of propranolol infusion and after the loading dose of propranolol to ensure that β-AR blockade did not influence lung function (34). Subjects performed isometric handgrip exercise at 30% of their MVC for 2 min while under β-AR blockade. At the end of the study, the same duration and volume of isoproterenol was again infused into the left arm while the maintenance dose of propranolol continued in the right arm to determine the effectiveness of our systemic β-AR blockade. Because of the high dose of propranolol given, vital signs were recorded at the end of the study in the seated and standing postures to ensure the subjects were not hypotensive. Handgrip exercise under control conditions (without isoproterenol and propranolol infusions) was performed on a separate day. The order of trials (control, propranolol) was conducted in a counterbalanced fashion and separated by at least 2 wk.

Data collection and statistical analysis. All variables were measured continuously and were analyzed offline. An average of the last 15 s of each minute is presented. RPP (the product of HR and systolic BP) was used as a noninvasive index of myocardial oxygen demand (24). CBV was used as an index of myocardial oxygen supply, and the ratio of ΔCBV to ΔRPP (i.e., the slope of the relationship between coronary flow and cardiac metabolism at peak exercise) was used as an index of myocardial oxygen supply-demand balance (19, 22).

For all variables, a two-treatment (drug, control) by two-time point (base, peak exercise) repeated-measures ANOVA was conducted using the raw physiological parameters (displayed in Fig. 2). Planned comparisons were used to compare physiological parameters at baseline and at the end of exercise (i.e., when it was expected that treatment effects would be most prominent). Changes from baseline (Δ) were also compared between treatments (displayed in Table 2). Student’s t-tests for independent samples were used to compare absolute values and changes from baseline between groups (young, older) (displayed in Tables 1 and 2). Physiological responses to isoproterenol were also compared between groups. All data are presented as means ± SE, and P values <0.05 were considered statistically significant.

RESULTS

Effect of propranolol and aging on physiological parameters at rest. Baseline data are shown in Table 1. Older men had lower height and weight (P < 0.047) compared with young men, but body mass index was not different between groups (P = 0.800). Young and older men had comparable resting systolic BP and HR (P > 0.3 for both) but older men had higher resting diastolic BP and mean arterial pressure (MAP) compared with young men (P < 0.03 for both).

The dose of propranolol did not vary between young and older men (56 ± 3 vs. 53 ± 2 mg, P = 0.360). Intravenous isoproterenol was well tolerated in all subjects. The HR response to intravenous isoproterenol was completely blocked by propranolol in both the young (pre: 28 ± 4 beats/min vs. post: 0 ± 1 beats/min, P < 0.001) and older subjects (pre: 29 ± 4 beats/min vs. post: 0 ± 1 beats/min, P < 0.001), indicating that β-AR blockade was effective. Propranolol did not alter pulmonary function in young men (FEV1 pre: 4.43 ± 0.62 l vs. post: 4.07 ± 0.41 l; and FVC pre: 5.60 ± 0.46 l vs. post: 5.31 ± 0.38 l, P > 0.671) or in older men (FEV1 pre: 3.19 ± 0.13 l vs. post: 3.11 ± 0.17 l; and FVC pre: 4.54 ± 0.20 l vs. post: 4.49 ± 0.35 l, P > 0.896). As expected, older men had lower FEV1 and FVC compared with young men (P < 0.041). As shown in Fig. 2, at rest, propranolol decreased HR and RPP (P < 0.05) in both young and older men. Propranolol tended to increase resting MAP (P = 0.057) in young men but not in older men (P = 0.455). Propranolol did not significantly affect resting CBV in young or older men (P > 0.05).
Effect of propranolol and aging on exercise-induced coronary hyperemia. Young men had higher MVC (46 ± 2 kg vs. 39 ± 1 kg, \( P = 0.019 \)) and RPE (15 ± 1 vs. 13 ± 1, \( P = 0.010 \)) compared with older men, but there was no difference in workload between groups (young: 14 ± 1 kg vs. older: 13 ± 1 kg, \( P = 0.341 \)). As shown in Fig. 2, isometric handgrip significantly increased MAP, HR, RPP, and CBV in both young and older men. Propranolol attenuated the HR and RPP responses to handgrip exercise in both groups. Whereas propranolol decreased the CBV response to handgrip exercise in young men, it had no effect on the CBV response in older men.

Table 2 shows cardiovascular responses to isometric exercise in young and older men under control conditions and propranolol. Under \( \beta \)-AR blockade, the \( \Delta \)HR at peak exercise was significantly lower (\( P = 0.031 \)), and \( \Delta \)CBV/RPP was higher (\( P = 0.030 \)) in older men compared with young men. Propranolol decreased \( \Delta \)CBV and the \( \Delta \)CBV/RPP ratio in young men (\( P < 0.048 \)) but did not alter these responses in older men (\( P > 0.293 \)).

Effect of isoproterenol and aging on coronary hyperemia. Older men required a greater dose of isoproterenol to increase their HR by 25 beats/min (16.4 ± 2.2 \( \mu \)g vs. 10.9 ± 0.7 \( \mu \)g, \( P = 0.040 \)). Because all subjects required a different dose (i.e., different infusion duration for each person), only the early
suggest that coronary hyperemia to low-dose isoproterenol. These data men but not in older men. In addition, older men had attenuated CBV at peak exercise was impaired by propranolol in young men; compared with the older men (8.2 ± 0.865). Figure 3 displays the baseline and peak CBV response to isoproterenol in one older man.

**DISCUSSION**

The purpose of this study was to investigate the contribution of β-ARs to coronary blood flow regulation in healthy humans. With respect to our hypothesis, we found that the change in CBV at peak exercise was impaired by propranolol in young men but not in older men. In addition, older men had attenuated coronary hyperemia to low-dose isoproterenol. These data suggest that β-ARs play a more prominent role in control of the coronary vasculature in young men than older men.

**β-AR contribution to CBV in atherosclerosis and aging.** Patients with atherosclerosis have impaired coronary vasodilation to physiological stressors (27, 30, 50, 59) and to intra-

coronary β-AR stimulation (3, 32, 47), but the role of β-ARs in the coronary circulation in healthy humans remains unclear. In healthy humans, β-ARs contribute to skeletal muscle vasodilation, which is blunted with age (37). One possible explanation for this phenomenon is that nitric oxide (NO) bioavailability decreases with age (53), and NO is important for β-AR-mediated vasodilation in skeletal muscle (7). NO also contributes to coronary vascular tone (48), and endothelium-dependent vasodilation of the coronary vessels is decreased with healthy aging (13), which may affect β-AR control of the coronary vessels.

It is well established that β-ARs contribute to coronary vascular responses in humans. Hodgson et al. (31) studied patients undergoing cardiac catheterization and demonstrated that intracoronary propranolol caused a reduction in CBV and a rise in coronary vascular resistance compared with saline injection. However, these patients had a mean age of 50, which prevents age comparisons. Recent studies show that coronary vasodilation is impaired in healthy older adults compared with young adults (41, 43), and the current study confirms and extends these findings by providing insight into the underlying mechanisms. Our data reveal that β-ARs contribute to coronary exercise hyperemia in young men, but this effect is greatly reduced with age. This observation may be due to changes in β-ARs with age, such as decreased sensitivity, downregulation of receptors, or altered downstream signaling. Alternatively, older men may have augmented α-adrenergic coronary vasoconstrictor responses during exercise compared with young men, which would dampen coronary vasodilation. However, we recently showed that coronary vasoconstriction to noxious forehead cooling was comparable between young and older adults (45). Whether α-adrenergic coronary vasoconstriction is enhanced in older men during exercise is unknown. Fitness levels may also account for differences in vascular control in our subjects. Although all subjects reported being physically active, we did not obtain any objective measures of physical fitness in these subjects, such as \( \text{VO}_{2\text{max}} \).

Although women were not included in the present study, it is plausible that β-AR control of the coronary vasculature is heightened in young women and attenuated in older, postmenopausal women compared with age-matched men. Young women have greater coronary exercise hyperemia compared with young men (43), which may be accounted for by heightened β-AR sensitivity, as observed in other studies (28, 36). Conversely, postmenopausal women display decreased β-AR-mediated vasodilation in the peripheral circulation compared with young women (28, 29), but comparisons to men are absent. Further studies are needed to investigate age-matched

---

**Table 2. Cardiovascular responses to isometric exercise in young and older men**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Older</td>
</tr>
<tr>
<td>ΔMAP, mmHg</td>
<td>21 ± 3</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>ΔHR, beats/min</td>
<td>12 ± 4</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>ΔRPP</td>
<td>2855 ± 53</td>
<td>2122 ± 528</td>
</tr>
<tr>
<td>ΔCBV, cm/s</td>
<td>9.7 ± 2.1</td>
<td>3.8 ± 1.3*</td>
</tr>
<tr>
<td>ΔCBV/RPP ratio</td>
<td>3.6 ± 0.9</td>
<td>2.0 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, HR, rate pressure product (RPP), and coronary blood flow velocity (CBV) are shown. *P < 0.05 compared with young men; †P < 0.05 compared with control condition.
sex comparisons in β-AR-mediated vasodilation and changes with healthy aging.

**β-ARs and coronary flow regulation.** It is well established that β-ARs contribute to coronary vasodilation in animals (10, 12, 16, 17, 25, 26, 39, 54). Studies have demonstrated that intravenous propranolol attenuates exercise-induced coronary hyperemia in swine (12, 17) and in canines (25). Myocardial β1-ARs indirectly contribute to coronary vasodilation by increasing myocardial oxygen demand (39). However, studies using selective β-AR antagonists reveal that vascular β1- and β2-ARs contribute to coronary vasodilation in vivo in swine (17) and in isolated canine cardiac preparations (54). In atherosclerotic vessels, intracoronary salbutamol (a selective β2-AR agonist) increased CBV (3, 47). However, whether vascular β1-ARs also modulate CBV in humans is unknown. Distinguishing β-AR subtypes in human coronary circulation was outside the scope of the present study. Future studies are needed to analyze the receptor subtypes that control the human coronary circulation.

**Effects of isoproterenol on CBV.** Isoproterenol is a nonselective β-AR agonist with a short half-life (<2 min) that has been previously used by our laboratory (44, 45) and others (5, 42, 49) to challenge the extent of β-AR blockade with propranolol. In the current study, intravenous propranolol completely blocked the tachycardia response to the peak dose of intravenous isoproterenol, indicating a full β-AR blockade. Consistent with previous studies (14, 52, 55, 58) in the prepropranolol state, the older men required a larger dose/longer infusion duration of isoproterenol to experience a ΔHR of 25 beats/min. These data suggest that older adults have lower myocardial β-AR sensitivity (predominantly β1-receptors). We also provide the novel finding that coronary vascular responses to isoproterenol were different between groups at 1.5 μg. Indeed, older men had ~50% less vasodilation in response to isoproterenol at this stage. These data in the coronary circulation are consistent with previous studies that revealed impaired forearm vasodilation in response to intra-arterial isoproterenol in older adults (46, 56) although age impairments in isoproterenol-induced vasodilation are not a universal finding (9, 35). There are a few issues that should be noted when evaluating the current CBV data. First, because the primary purpose of isoproterenol in this study was to raise HR by 25 beats/min (i.e., to challenge the β-AR blockade), all subjects received a different dose of isoproterenol. Therefore, we were only able to compare the early stages in which all subjects had received the same dose. Second, the volume of infusate was very low, and each stage was only 1 min in duration so that the physiological effects at 0.5 μg and 1.0 μg were minimal. Third, the two groups of men had different body mass. Because lean body mass and blood volume also differ between young and older subjects (15, 33), a future approach will be to infuse isoproterenol based on lean body mass. In the current study, the older men had lower body mass (and presumably less lean body mass), so they actually received greater doses of isoproterenol per blood volume; matching the groups for blood volume or body fat would have likely magnified group differences in CBV.

**Conclusion.** This study may explain why aging is a risk factor for cardiac ischemia. Similar to patients with coronary artery disease (3, 32, 47), we found that healthy older men also have impaired coronary hyperemia to β-AR stimulation and that β-AR blockade with propranolol does not alter their coronary vascular responses. These data indicate that β-AR control of coronary exercise hyperemia is decreased in healthy older men. Further studies are needed to investigate specific adrenergic receptor subtypes that contribute to coronary exercise hyperemia in healthy humans and changes with age that may contribute to the development of atherosclerosis and heart disease.

**ACKNOWLEDGMENTS**

We thank Cheryl Blaha, Jessica Mast, and Aimee Cauffman for nursing support; Dr. Michael Herr for engineering support; Matt Hefferman for technical assistance; Anne Muller for preparing the graphics for this study; and Kris Gray and Jen Stoner for administrative support.

**GRANTS**

This work was supported by the National Institutes of Health Grants P01 HL096570 and U1L TR000127 (both to L. Sinoway) and R01 HL098379 (to U. Leuenberger).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


