Similarity between carotid and coronary artery responses to sympathetic stimulation and the role of alpha-1 receptors in humans

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Short title: SNS activation & central artery responses

WORD COUNT: 5053

ABSTRACT WORD COUNT: 250

FIGURES: 4

TABLES: 2

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KEY POINTS SUMMARY

- Sympathetic activation using the cold pressor test (CPT) leads to marked vasodilation in coronary arteries, whereas previous work also demonstrated dilation in carotid arteries.
- We examined whether sympathetic activation, through the CPT and lower-body negative pressure (LBNP), leads to similarity in response in the coronary and carotid artery, whilst we also assessed the role of α_1-receptors in these responses.
- We showed that changes in carotid and coronary responses were positively related, with both arteries demonstrating dilation during CPT, whereas constriction is present in both arteries during LBNP. Furthermore, α_1-receptor blockade abolished CPT-induced changes, whilst LBNP responses remained in both arteries.
- Our results strongly indicate presence of strong similarity between carotid and coronary responses to sympathetic tests, with between-artery agreement in the direction of change as well as the role of α_1-receptors mediating these responses.

ABSTRACT

Background. Coronary and common carotid (CCA) arteries in healthy subjects dilate in response to sympathetic activation. To better understand similarity between these arteries, we examined coronary and carotid artery responses to different sympathetic tests, and explored the role of α_1-receptors in mediating these vasomotor responses.

Methods. In a randomised order, 10 healthy participants (25±3 yrs) underwent sympathetic stimulation using either the cold pressor test (CPT; 3-minutes left hand immersion in ice-slush) or lower-body negative pressure (LBNP). Before and during sympathetic tests, CCA diameter and velocity (Doppler ultrasound) and left anterior descending (LAD) coronary artery velocity (echocardiography) were recorded across 3-min. Measures were repeated 90-min following selective α_1-receptor blockade via oral Prazosin (0.05mg per kg body weight).

Results. CPT significantly increased CCA diameter, LAD maximal velocity and velocity-time integral area-under-the-curve (all $P<0.05$). In contrast, LBNP resulted in a decrease in CCA diameter, LAD maximal velocity and VTI (all $P<0.05$). Changes in CCA diameter and LAD VTI-responses to sympathetic stimulation were positively related ($r=0.66$, $P<0.01$). Following the α_1-receptor blockade, the CCA and LAD velocity responses to CPT were abolished. In contrast, during LBNP (-30 mmHg), α_1-receptor blockade did not alter CCA or LAD responses.

Conclusion. Carotid and coronary arteries demonstrate dilation during CPT, whereas both show constriction during LBNP. Following α_1-receptor blockade, CPT-induced changes were abolished, whilst LBNP responses were unaltered. These data indicate strong similarity between CCA and LAD responses to sympathetic tests, with between-artery agreement in the direction of change as well as the role of α_1-receptors mediating these responses.

KEYWORDS: carotid artery, coronary artery endothelial function, sympathetic nervous system, cardiovascular disease, α1-adrenoceptors

ABBREVIATION LIST

Cardiovascular disease (CVD)

Cardiac output (CO)

Cold pressor test (CPT)

Common carotid artery (CCA)

Diastolic blood pressure (DBP)

Heart rarate (HR)

Left anterior descending (LAD)

Left anterior descending artery, mean diastolic velocity (LADVmean)

Left anterior descending artery, peak diastolic velocity (LADVmax)

Lower body negative pressure (LBNP)

Partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$)

Partial pressure of end-tidal oxygen ($P_{ET}O_2$)

Rate pressure product (RPP)

Systolic blood pressure (SBP)

Sympathetic nervous system (SNS)

Stroke volume (SV)

INTRODUCTION

Impairment of coronary artery blood flow and endothelial function characterizes the development of cardiovascular disease (CVD). Activation of the sympathetic nervous system (SNS) is an important and clinically-relevant prognostic stimulus (Feigl, 1987; Schachinger *et al.*, 2000). In the coronary arteries, SNS activation can result in a vasoconstrictor (via α_1-receptors) or vasodilatory response (via the α_2-, and β-receptors)(Barbato, 2009). Whilst vasodilator pathways prevail in healthy volunteers - leading to significant coronary dilation (Doucette *et al.*, 1992; Momen *et al.*, 2009) - experimental studies in patients with coronary artery disease demonstrate that stimulation of the SNS mediates vasoconstriction(Nabel *et al.*, 1988; Zeiher *et al.*, 1989). Importantly, coronary artery responses to SNS activation independently predict future cardiovascular events (Schachinger *et al.*, 2000; Nitenberg *et al.*, 2004b). These latter studies highlight the potential clinical relevance of examining SNS-induced coronary artery responses. However, serious disadvantages of these measurements include difficulty of assessment and invasiveness of examining coronary arteries.

Evidence that responsiveness of the larger (and more accessable) peripheral arteries may serve as a surrogate measure for coronary artery vasomotor function (Anderson *et al.*, 1995) is supported by close correlation of coronary and forearm artery responses to elevation in shear and/or infusion of vasodilator drugs (Takase *et al.*, 1998; Takase *et al.*, 2005). Although direct comparisons are lacking, several studies have reported no change in peripheral (i.e., brachial artery) conduit artery diameter upon SNS-activation, suggesting distinct responses between coronary and peripheral conduit arteries to SNS-activation (Harris *et al.*, 2000; Lind *et al.*, 2002; Dyson *et al.*, 2006; Thijssen *et al.*, 2006; Momen *et al.*, 2009). Interestingly, previous work demonstrated dilation of the more central carotid artery during sympathetic activation (via cold pressor test, CPT) in patients with (risk for) cardiovascular disease and healthy

subjects (Rubenfire *et al.*, 2000; van Mil *et al.*, 2016). To date, no previous study directly compared vasodilator responses between carotid and coronary arteries to distinct SNS activation stimuli. Furthermore, although the vasoconstrictor responses of the coronary arteries via α_1-receptors have been well documented in humans and animals (Mohrman & Feigl, 1978; Heusch *et al.*, 1984; Kern *et al.*, 1985), little is known about whether α_1 – pathways regulate *both* the coronary and carotid arteries *during* SNS activation in humans.

Our first aim is to examine whether activation of the SNS, either through the CPT (i.e. a valid test to elevate SNS activity and blood pressure)(Hines & Brown, 1936; Victor *et al.*, 1987) or lower-body negative pressure (LBNP, test that elevates SNS activity, with preserved blood pressure [at least during mild to moderate negative pressures])(Victor & Leimbach, 1985; Jacobs *et al.*, 1996), lead to vasodilation in both the coronary and carotid artery. Our second aim was to assess the role of α_1-receptors to these responses by using an oral, selective α_1-receptor blocker (i.e. Prazosin). Based on previous work (van Mil *et al.*, 2016), we hypothesise that SNS activation will lead to an increase carotid artery diameter and left anterior descending coronary artery velocity (LAD velocity), independent on the test of SNS activation. Furthermore, based on the vasoconstrictive nature of α_1-receptors, we anticipated that systemic blockade of α_1-receptors would exaggerate the vasodilator responses to activation of the SNS (i.e. greater increase in CCA diameter and increase in LAD velocity).

METHODS

Ethical approval

This study was approved by the Human Ethics Committee of the University of British Columbia and conformed to the standards set by the Declaration of Helsinki. All volunteers provided written informed consent.

Participants

We recruited 10 healthy male participants (mean age 25±3 years, height 1.78±0.1 m, and weight 76±9 kg). Exclusion criteria were a history of cardiovascular disease (i.e. angina pectoris, myocardial infarction, heart failure), lung disease (i.e. COPD, lung cancer), brain disease (i.e. stroke, dementia), presence of Raynaud's phenomenon, scleroderma, chronic pain and/or open wounds on the upper extremities, obesity (body mass index >30 kg/m^2), diabetes mellitus type 1 or 2, history of smoking, or elevated blood pressure (systolic >130 mmHg; diastolic >85 mmHg).

Experimental design

All participants reported to our laboratory for a single visit. They were asked to abstain from strenuous exercise for 24 hours and abstain from dietary products known to affect endothelial function for ≥18 hours prior to the testing session (i.e. vitamin C, caffeine and alcohol). Moreover, participants were asked to fast for ≥2 hours, adapted from existing guidelines to assess peripheral vascular function (Thijssen *et al.*, 2011). Participants rested in the supine position for >15 minutes on a bed in a temperature-controlled room (23±1°C). Subsequently, participants underwent LBNP and two CPT, in a randomly assigned order, with 45-minutes rest between tests. All tests involved simultaneous assessment of common carotid artery (CCA) diameter and velocity (ultrasound) and left anterior descending (LAD) coronary artery velocity (echocardiography) before (across a 1-minute baseline) and during sympathetic stimulation. The protocol was repeated 90-minutes after oral administration of Prazosin (i.e. α_1- adrenergic receptor antagonist, 0.05mg per kg body weight)(Jones *et al.*, 2011; Atkinson *et al.*, 2015).

Experimental measures

Common carotid artery diameter and velocity. Left carotid artery diameter and red blood cell velocity were recorded simultaneously and continuously during baseline (1-minute) and sympathetic stimuli (i.e. 3-minutes CPT, and ~18-minutes LBNP). Carotid artery image acquisition was performed using a 10-MHz multifrenquency linear array handheld probe attached to a high resolution ultrasound machine (15L4, Terason T3200, Burlington, MA, USA). When an optimal image was found, 2-3 cm proximal from the bifurcation, the probe was held stable and the ultrasound parameters were set to optimise the longitudinal, B-mode image of the lumen-arterial wall interface. Continuous pulsed wave Doppler velocity assessments were also obtained and were collected at the lowest possible insonation angle (always <60º). Assessment was performed by an experienced sonographer (ACCM), whom has a hour-to-hour reproducibility (i.e. coefficient of variation) of CCA baseline diameter of 5% and reproducibility of 6% for the CCA diameter response to the cold pressor test (van Mil *et al.*, 2016).

Coronary artery velocity. Before and during both CPT and LBNP, the left anterior descending (LAD, cm) coronary artery velocity was examined using transthoracic ultrasound. This assessment was performed simultaneously with CCA diameter and velocity responses. All echocardiographic measurements were collected by a trained sonographer (MS) on a commercially available ultrasound system (Vivid E9; GE, Fairfield, CT) using a broadband M5S 5 MHz or a 3V 3D-array transducer. Participants assumed a left lateral position to allow for data collection. The LAD was imaged using a modified parasternal short axis view from the fourth or fifth left intercostal space, and was assessed using pulsed-wave Doppler. The transducer was positioned such that a 2- to 3-mm segment of the LAD was imaged along the long axis, taking care to align the pulse-wave cursor with the length of the vessel. With a

sample volume (2.0 mm) positioned over the color Doppler signal in the LAD, measurements of the LAD velocity were collected during the sympathetic tests.

Blood pressure and heart rate. All continuously recorded cardiovascular measurements were acquired at 200 Hz using an analog-to-digital converter (Powerlab/16SP ML 880; ADInstruments, Colorado Springs, CO, USA) interfaced with a personal computer. Before and during CPT and LBNP, systolic and diastolic blood pressure (SBP and DBP, in mmHg, respectively), stroke volume (SV, ml), rate-pressure product (RPP, HR x SBP, a reliable indicator for myocardial oxygen demand)(Gobel *et al.*, 1978), and cardiac output (CO, L/min) were continuously measured using non-invasive finger photoplethysmography (Finometer Pro, Finapres medical systems, Amsterdam, Netherlands). Heart rate (HR, beats per minute) was recorded using three-lead electrocardiography, placed in lead II configuration (Bioamp, ML132, ADInstruments, Colorado Springs, CO, USA).

Sympathetic stimuli

Cold pressor test. The cold pressor tests (CPT) consisted of a 3-minute immersion of the left hand in a bucket of ice slush (~4.0°C)(van Mil *et al.*, 2016). The participant was positioned in supine position on a tilt bed, tilted slightly to the left lateral position (~25-30°), to facilitate arm movement in the bucket of slush without significant movement of the body, and provide adequate coronary assessment. After a 1-minute baseline period, the participants hand was immersed up to the wrist in the ice-slush for 3 minutes. The participant was instructed remain quiet during the CPT to provide for valid CCA assessment. The partial pressures of end-tidal carbon dioxide ($P_{ET}CO_2$) and oxygen ($P_{ET}O_{2)}$ were clamped at baseline values for the entire duration of the protocol to reduce the potential impact of hyperventilation on the vascular responses, upon an end-tidal forcing approach described extensively elsewhere (Tymko *et al.*,

2016). To reduce measurement error, CPT procedures were repeated twice and averaged for analyses (van Mil *et al.*, 2016).

Lower body negative pressure. The participant was positioned in the supine position on a tilt bed, and strapped into a custom-made airtight, lower-body suction chamber at the level of the iliac crest (Tymko, 2016). The LBNP chamber was then moved from supine position into a left lateral position (~25-30°) to ensure adequate coronary imaging. The lower body negative pressure test consisted of a 5-minute baseline, followed by progressive 2-minute stages, using increments of -10 mmHg, to -80 mmHg or until pre-syncope. LBNP was terminated when *a*) pre-syncope, defined by a sustained drop in systolic blood pressure <80 mmHg for more than 10 seconds, occurred (Lewis *et al.*, 2014), or *b*) on participants request due to the onset of subjective symptoms (e.g. feelings of dizziness, nausea, faintness). During the Prazosin condition, participants were unable to last longer than -40mmHg during the LBNP test. For reliable comparison between the control and drug condition, we chose to only include data until -30mmHg.

Data analysis

Carotid artery responses. Analyses of diameter (cm), blood flow (ml/sec), blood velocity (cm/sec) and shear (s^{-1}) were performed using custom-designed edge-detection and wall-tracking software, which is largely independent of investigator bias, as was extensively described elsewhere (Black *et al.*, 2008). Baseline diameter, blood flow, blood velocity and shear were calculated as the mean of data acquired across a 1-minute baseline period. For the CPT, data were calculated for 10-second intervals. LBNP data was calculated per 1-minute intervals. Subsequently, offline image analysis involves the identification of the region of interest (ROI), to allow for automated calibration on the B-mode image and velocities on the

Doppler assessment (Thijssen *et al.*, 2009). A ROI is drawn around the optimal B-mode image, in which a pixel-density algorithm automatically identifies the near- and far wall. Another ROI is drawn around the Doppler waveform, which is synchronized with the B-mode diameter ROI. Ultimately, this allows for blood flow and shear rate calculations (Thijssen *et al.*, 2009). Peak diameter change was calculated relative to baseline diameter.

Coronary artery responses. All images were exported for offline analysis using commercially available software (EchoPAC Version 13.0; GE Medical, Horten, Norway). All echocardiographic values represent an average value of three cardiac cycles representing the clearest of five collected images for each experimental stage. The collected waveforms were analyzed to determine mean diastolic velocity (LADVmean, cm/s), peak diastolic velocity (LADVmax, cm/s), and the velocity time integral (VTI, cm). Participants in whom at least 1 image was suboptimal, were excluded prior to analyses (Boulet *et al.*, 2016).

Blood pressure and heart rate. Analyses of systolic-, and diastolic pressure, heart rate, cardiac output, stroke volume, and the rate-pressure product (RPP) were performed in commercially available software (LabChart V7.1, ADInstruments, Colorado Springs, CO, USA). Measurements were averaged per 10-second bins for analyses for the CPT, and 1-minute bins for the LBNP analyses. Baseline CPT was averaged over a 3-minute period. Continuous blood pressure measurements were calibrated to automated brachial blood pressure readings during baseline (HEM-775CAN, Omron Healthcare, Bannockburn, IL, USA).

Statistical analyses

All data were presented as mean ± SD unless stated otherwise. Parameters were tested for normality using a Shapiro-Wilk test. Responses of both the CCA (i.e. diameter, blood velocity, flow and shear) and LAD (i.e. mean velocity, max velocity and VTI) were assessed during the sympathetic stimulus with paired Students' t-tests, and over time with repeated

measurement ANOVA's (missing values were only imputed based on previous and consecutive measurements when available). Whether CCA and LAD changes in diameter, velocity, flow and shear to the sympathetic stimuli (i.e. 'time') differed between conditions (i.e. control *vs* Prazosin) were examined using a repeated measure 2-way ANOVA, with Sidak correction to account for multiple comparisons. Data were analysed using SPSS 20.0 software (IBM SPSS, IBM Corp., Armonk, NY, USA). Values for $p<0.05$ were assumed to be statistical significant.

RESULTS

Carotid vs coronary artery responses: different stimuli.

Cold pressor test. The CPT caused a significant increase in systolic and diastolic blood pressure and the rate-pressure product (RPP), whilst no change was found in stroke volume, heart rate and cardiac output (n=9, Table 1). Although the diameter of the CCA increased (2%) significantly during CPT ($P<0.001$, Figure 1), CCA velocity, flow and shear rate did not change significantly across time during CPT ($P>0.05$, data not shown due to space restrictions). LAD showed a significant increase in maximum velocity (n=6, baseline 0.25±0.03 to peak 0.34±0.02, $P<0.05$) and VTI ($P<0.05$, Figure 1) during CPT.

Lower body negative pressure. LBNP caused a gradual, but significant increase in heart rate, a decrease in stroke volume and diastolic blood pressure, whilst systolic blood pressure and cardiac output were preserved (n=9, Table 2). LBNP caused a significant decrease (-0.8%) in CCA diameter (Figure 2), whereas no changes were found in CCA velocity, flow and shear (data not shown). Likewise, during LBNP, LAD maximum velocity (n=5, Table 2) and peak VTI (Figure 2) were both reduced. When the CPT- and LBNP-induced changes were pooled

(n=20), a significant correlation was found between changes in CCA diameter and LAD peak VTI (r=0.65, $P<0.01$).

Carotid vs coronary artery response to sympathetic activation: role of α-receptors

Cold pressor test. Prazosin attenuated the increase in blood pressure during CPT, and resulted in a larger increase in cardiac output and RPP during the CPT (n=9, Table 2); stroke volume and heart rate responses were unaltered (Table 2), neither were CCA flow, shear and velocity (data not shown). Following Prazosin administration at baseline, both CCA diameter and higher LAD VTI were elevated (n=6, Figure 3). Prazosin significantly abolished the increases in CCA diameter (Figure 3A) and LAD peak VTI during the CPT (Figure 3C-D). Likewise, Prazosin also abolished the CPT-induced increase in LAD mean and maximum velocity (Table 2).

Lower-body negative pressure. Prazosin exaggerated the increase in heart rate and RPP during LBNP, whilst blood pressure decreased during the Prazosin trial (n=9, Table 2). Baseline CCA diameter, flow and velocity were significantly larger following Prazosin adminstration (0.684±0.05 to 0.706±0.05, 10.2±1.6 to 12.0±2.3, and 27.6±5.0 to 30.0±5.5, respectively, all $P<0.05$). In contrast, no significant difference was found for baseline LAD velocity (n=5, Figure 4). Prazosin did not alter the CCA diameter or LAD peak VTI or peak velocity responses during LBNP (up to -30 mmHg; Table 2, Figure 4).

DISCUSSION

We present the following two novel findings. First, activation of the SNS using the CPT significantly increased CCA diameter and coronary artery velocity. In contrast, SNS activation using LBNP mediated a decrease in both CCA diameter and coronary artery

velocity. Despite a divergent response to both sympathetic tests, we found a positive relationship between coronary and carotid responses to sympathetic stimulation. Second, systemic blockade of the α_1-receptors significantly attenuated the dilator response of both the carotid and coronary arteries during the CPT, whilst these changes were unaltered during LBNP. Taken together, we found divergent responses to SNS activation, whilst apparent similarity between carotid and coronary arteries in vascular responses to sympathetic stimulation is present, and the role of α_1-receptors in mediating these responses seems comparable between arteries. Our observations have clinical relevance, as the common carotid artery may serve as an easy and accessible surrogate for coronary responses to sympathetic stimulation.

Activation of the SNS using a CPT is well known to induce marked coronary artery vasodilation in healthy volunteers (Nabel *et al.*, 1988; Zeiher *et al.*, 1989). Similarly, in our group of healthy subjects, we found an increase in LAD VTI during CPT. Previous studies found strong agreement between increases in coronary artery blood velocity and blood flow in response to sympathetic stimulation (Doucette *et al.*, 1992; Momen *et al.*, 2009; Monahan *et al.*, 2013), suggesting that the increase in LAD VTI can be interpreted as the presence of vasodilation. The CPT also resulted in a dilation in the CCA, suggesting similarity between both central artery responses to the CPT. Interestingly, these dilator responses markedly contrast with peripheral artery responses, since brachial or superficial femoral arteries demonstrate negligible diameter changes during CPT (Lind *et al.*, 2002; Dyson *et al.*, 2006). Central, eleastic arteries may thus respond differently to SNS activation using the CPT compared to muscular, peripheral arteries. This notion is further supported by observations of abdominal aorta dilation during the CPT (Chandraratna *et al.*, 2009).

We also examined coronary and carotid artery responses to LBNP; a test known to increase SNS activation, but with blood pressure being maintained around baseline level (Victor & Leimbach, 1985). In contrast to the CPT, reduction in coronary LAD velocity and a decrease in carotid artery diameter was observed during LBNP. Nonetheless, the presence of coronary artery vasoconstriction during LBNP is in agreement with a previous study that reported a reduction in LAD coronary blood velocity during comparable LBNP levels of -10 and -30 mmHg LBNP (Momen *et al.*, 2009). Distinct artery responses to different tests of sympathetic activation have also been reported in peripheral conduit arteries (Dyson *et al.*, 2006). Potentially, CPT and LBNP mediate sympathetic activation through different pathways, leading to distinct vascular responses in peripheral and central arteries. Whilst CPT causes an immediate increase in blood pressure (Dyson *et al.*, 2006; Momen *et al.*, 2009), LBNP mediates a gradual, baroreflex-mediated activation of the sympathetic nervous system that preserves blood pressure. Both sympathetic tests also demonstrate distinct time-dependent changes in circulating catecholamines. For example, there is an immediate elevation in epinephrine and norepinephrine after CPT, whereas elevation in epinephrine and norepinephrine during LBNP seem time- and intensity-dependent (Robertson *et al.*, 1979; Jacobs *et al.*, 1996; Dyson *et al.*, 2006; Momen *et al.*, 2009). Although further research is warranted to better understand the distinct vascular responses to different sympathetic tests, our data during LBNP provides further evidence that both the carotid and coronary arteries demonstrate similar responses, independent of the sympathetic test.

Under physiological conditions, α_1-receptors mediate vasoconstriction in the coronary arteries during a sympathetic stimulus (Mudge *et al.*, 1976; Kern *et al.*, 1985). Indeed, α_1-receptor blockade has been associated with an increase in baseline LAD velocity as well as an increase in baseline CCA diameter, velocity and blood flow. However, in contrast to our hypothesis,

α_1-blockade attenuated the dilator responses of coronary and carotid arteries during activation of the SNS using the CPT. One potential explanation is that the increase in baseline diameter and/or flow (induced by α_1-receptor blockade) prevented a further increase upon SNS stimulation. Previous work in peripheral arteries repeatedly demonstrated the presence of a strong, inverse relation between baseline diameter and endothelium-dependent and –independent vasodilator responses (Silber *et al.*, 2001; Thijssen *et al.*, 2008). In contrast, however, our data does not reveal such a relation between resting diameter and peak responses in the carotid artery diameter (CPT control r=-0.280, Prazosin r=-0.275, LBNP control r=-0.401, Prazosin r=-0.219, all P>0.05). Furthermore, the increase in resting diameter was well below the peak response observed during the CPT. Therefore, although we acknowledge the limations of correlational inferences, CCA and LAD dilation during α_1-blockade at baseline does not seem to explain the attenuated vasomotor responses to CPT.

Another potential explanation for our observations may relate to the pharmacological actions of α_1-receptor blockers. In healthy coronary arteries, vasoconstriction upon sympathetic stimulation is largely mediated via α_1-receptors, whilst α_2-receptors play a minor role in these responses (Baumgart *et al.*, 1999). However, previous studies in both animals and humans found that during α_1-receptor blockade, SNS activation causes constriction of coronary arterioles which is mediated via activation of α_2-receptors (Chilian, 1991; Indolfi *et al.*, 1992; Heusch *et al.*, 2000). Accordingly, blockade of the α_1-receptors in our study may have yielded stimulation of α_2-receptors *during* activation of the SNS using the CPT. Consequently, the vasodilator responses may be affected by the constrictive actions of the α_2-receptors during the CPT. This hypothesis needs further exploration.

Clinical relevance. Coronary artery responsiveness to SNS stimulation, including the CPT, has shown a strong predictive ability for future cardiovascular disease and/or events (Schachinger *et al.*, 2000; Nitenberg *et al.*, 2004a; Nitenberg *et al.*, 2004b). The similarity in vasomotor responsiveness between coronary and carotid arteries suggests that the carotid artery may serve as a surrogate measure for coronary vascular responses to SNS stimulation. An important advantage of measuring the carotid artery is its easy accessibility. This warrants future studies to further explore the potential clinical use of examining CCA responses to SNS stimulation.

Methodological considerations. The rate-pressure product, an indirect measure of myocardial oxygen demand, increased in both tests, whilst α_1-receptor blockade caused a further increase. This increase in RPP, and thus increase in myocardial work effort, may interfere with direct responses of the coronary vasculature to sympathetic stimuli. However, due to the indirectness of the measurement of myocardial workload (Boulet *et al.*, 2016), it remains to be clarified to what extend this influenced our results. A strength of our study was that we controlled for end-tidal gases at baseline values, during both CPT and LBNP, and the α_1-receptor blockade condition. Fluctations and alterations in $P_{ET}CO_2$ are known to directly influence the diameter of the CCA (Sato *et al.*, 2012) and LAD VTI (Boulet *et al.*, 2016). Following our alpha-1 blockade, which directly affects mean arterial pressure and ventilatory regulation during sympathetic activation, clamping $P_{ET}CO_2$ and $P_{ET}O_2$ to baseline values reduced the possible interference with our carotid and coronary artery responses. A limitation in our study was that subjects reached pre-syncope at -30 or -40 mmHg when LBNP was combined with Prazosin. Given the gradual changes in vasomotor responses during the LBNP, we could only compare data between both sessions up to -30 mmHg. Another limitation was that, due to the suction of the LBNP box, movement of the participants

prevented valid assessment of coronary responses in 5 participants. Whilst these limit the ability for detailed assessment of the role of the α-receptors in large sample, data clearly demonstrates similarity between coronary and carotid artery responses.

To summarize, despite sympathetic tests mediating distinct vascular responses, our data demonstrates strong similarity between coronary and carotid artery reactivity in response to the cold pressor test (i.e. dilation) and lower body negative pressure (i.e. constriction). Additionally, blockade of the α_1-receptors significantly attenuated the dilator responses in both arteries during the CPT, whilst no changes were found during LBNP, suggesting a similar role for α-receptors to contribute to vasomotor responses in coronary and carotid arteries. The similarity between carotid and coronary arteries may have future clinical relevance when using the carotid artery as a surrogate for coronary arteries.

ADDITIONAL INFORMATION

Acknowledgements

We would like to thank Kevin Wildfong for his valuable contribution to the experiments, and Glen Foster for providing the necessary equipment and laboratory space.

Author contributions

DHJT, ACCMM, MT and PA designed the study. ACCMM, MS, MT and TK were involved in data collection and analyses. ACCMM and DHJT performed the statistical analyses. All authors contributed to the interpretation of the data, writing of the manuscript. All authors provided approval of the final version and agreed to be accountable for all aspects of the work. All persons designated as authors qualify for authorship and all those who qualify for authorship are listed.

Sources of funding

Philip N. Ainslie, and the experimental studies which were conducted in his laboartory, was supported by a Canadian Research Chair in Cerebrovascular Physiology, a Natural Sciences and Engineering Research Council Discovery Grant and Canadian Foundation of Innovation Grant. MT is funded by a Natural Sciences and Engineering Research Council Doctoral award. DJG is supported by a National Health and Medical Research Council (NHMRC) Principal Research Fellowship (APP1080914). ACCM is financially supported by a Top Institute for Food and Nutrition-grant.

Disclosures

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

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FIGURES

FIGURE 1. Responses of the carotid artery and the LAD coronary artery to CPT. Data are presented as mean, error bars represent standard error of the mean (SEM). White bars represent baseline measurements, black bars represent peak values. A. Diameter change of the carotid artery over time. B. Percentage change in carotid diameter at baseline and during CPT (area under the curve, AUC). C. VTI change of the LAD coronary artery over time. D. Percentage change in LAD coronary artery VTI at baseline and during CPT (area under the curve, AUC).

FIGURE 2. Responses of the carotid artery and the LAD coronary artery to LBNP. Data are presented as mean, error bars represent standard error of the mean (SEM). White bars represent baseline measurements, black bars represent peak values. A. The diameter change of the carotid artery over time. B. The percentage change in carotid diameter at baseline and during LBNP. C. the VTI change of the LAD coronary artery over time. D. The percentage change in LAD coronary artery VTI at baseline and during LBNP.

FIGURE 3. Responses of the carotid artery and the LAD coronary artery to CPT, Control *versus* Prazosin condition. Data are presented as mean, error bars represent standard error of the mean (SEM). White bars represent the *Control* condition, black bars represent the *Prazosin* condition. A. Diameter change of the carotid artery over time. B. Percentage change in diameter. C. VTI change of the LAD coronary artery over time. D. Percentage change in LAD coronary artery VTI at baseline and during the CPT.

FIGURE 4. Responses of the carotid artery and the LAD coronary artery to LBNP, Control *versus* Prazosin condition. Data are presented as mean, error bars represent standard error of

the mean (SEM). White bars represent the *Control* condition, black bars represent the *Prazosin* condition. A. Diameter change of the carotid artery over time. B. Percentage change in carotid diameter at baseline and during the LBNP. C. VTI change of the LAD coronary artery over time. D. Percentage change in LAD coronary artery VTI at baseline and during the LBNP.

FIGURE 1.

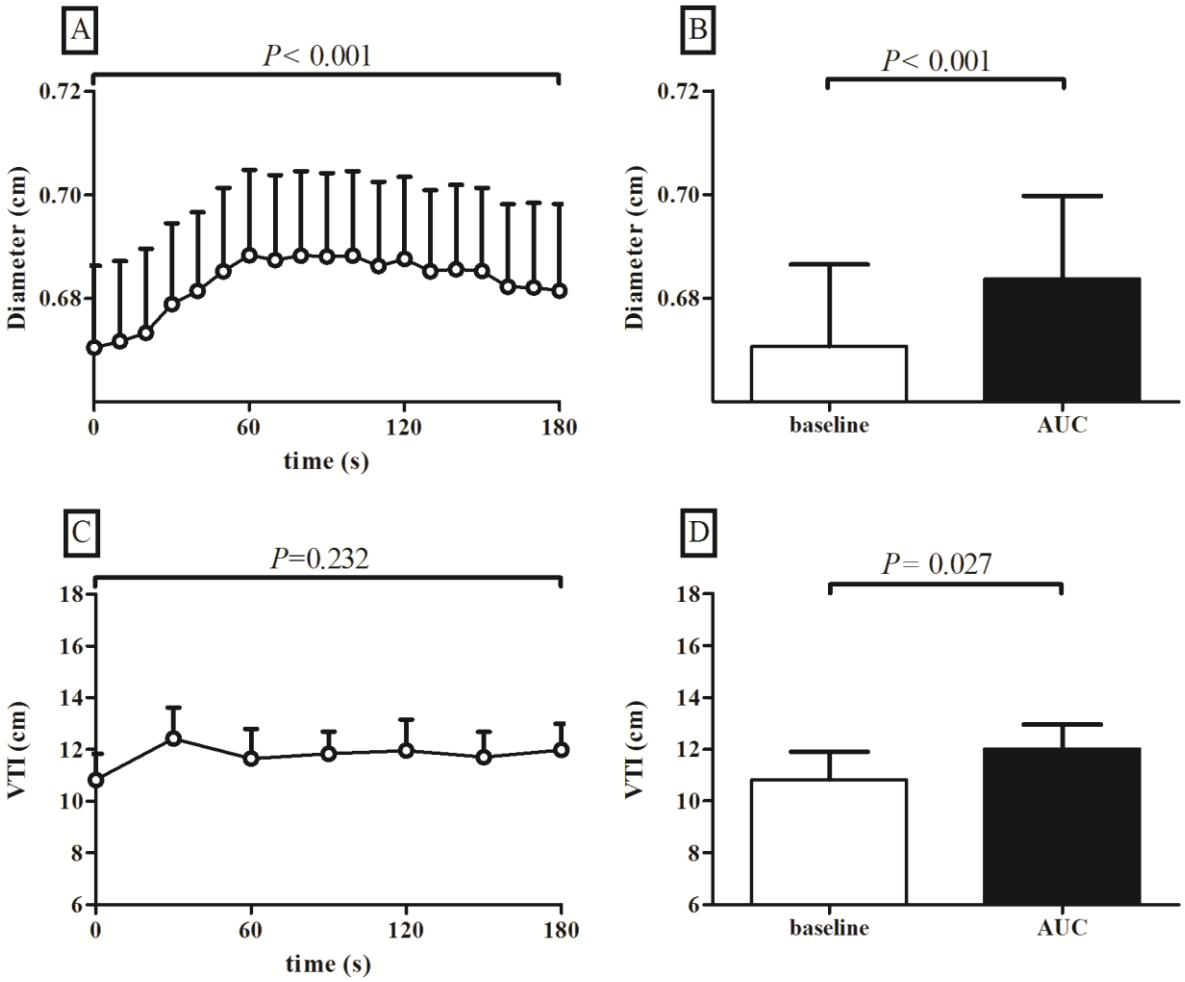
A
P< 0.001
0.72
0.70
0.68
Diameter (cm)
0
60
120
180
time (s)
B
P< 0.001
0.72
0.70
0.68
Diameter (cm)
baseline
AUC
C
P=0.232
18
16
14
12
10
8
6
VTI (cm)
0
60
120
180
time (s)
D
P= 0.027
18
16
14
12
10
8
6
VTI (cm)
baseline
AUC

FIGURE 2.

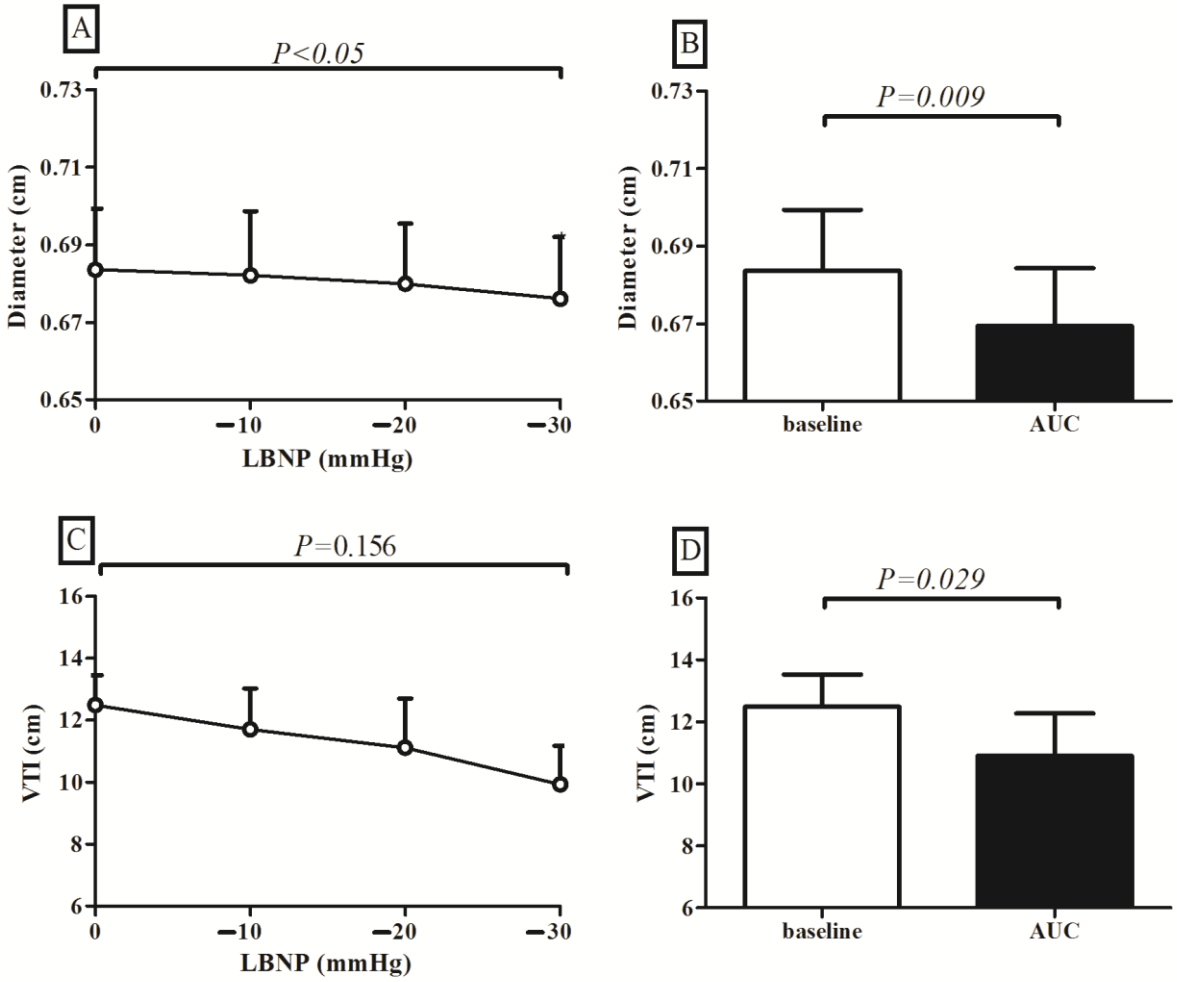
A
P<0.05
Diameter (cm)
0.73
0.71
0.69
0.67
0.65
0
−10
−20
−30
LBNP (mmHg)
B
P=0.009
Diameter (cm)
0.73
0.71
0.69
0.67
0.65
baseline
AUC
C
P=0.156
VTI (cm)
16
14
12
10
8
6
0
−10
−20
−30
LBNP (mmHg)
D
P=0.029
VTI (cm)
16
14
12
10
8
6
baseline
AUC

FIGURE 3.

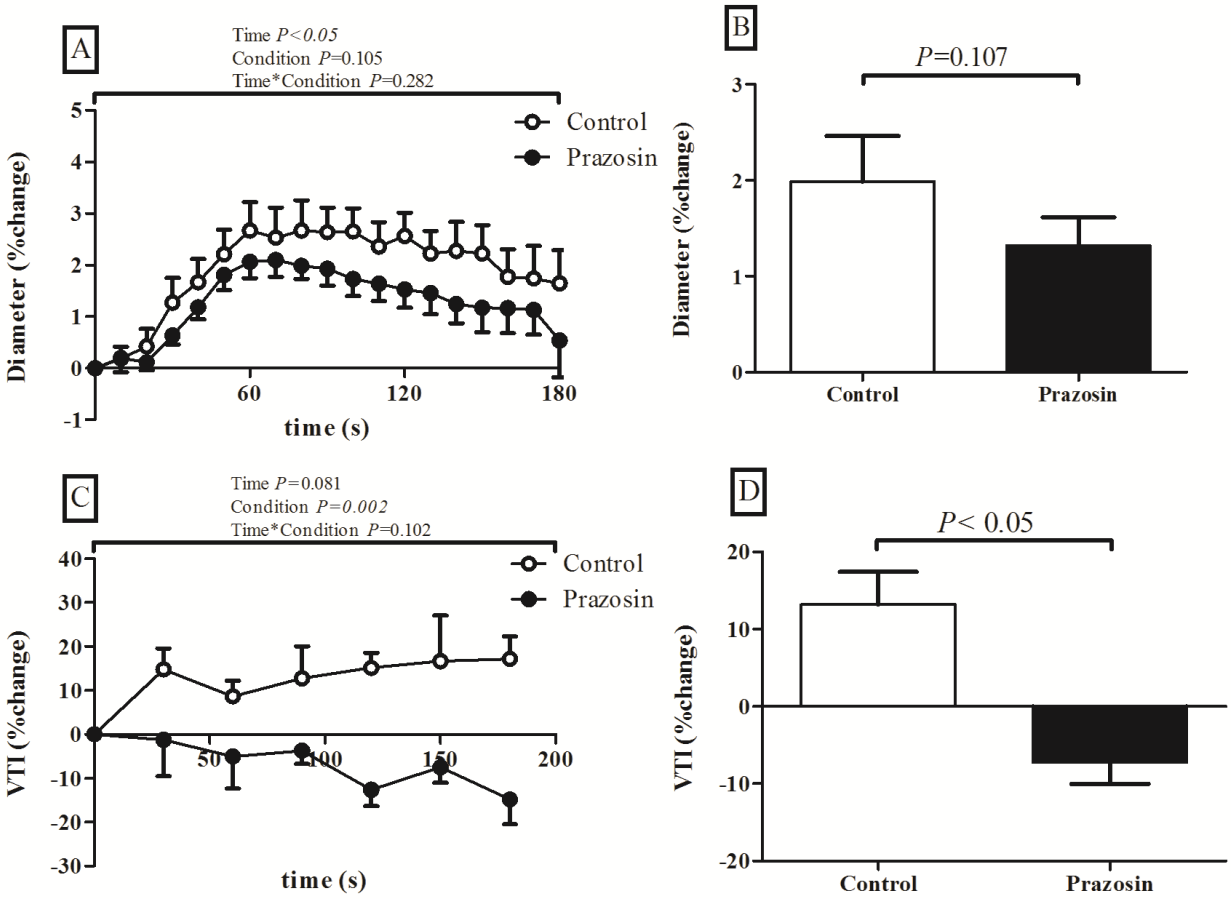
A
Time P<0.05
Condition P=0.105
Time*Condition P=0.282
Control
Prazosin
Diameter (%change)
time (s)
B
P=0.107
Diameter (%change)
Control
Prazosin
C
Time P=0.081
Condition P=0.002
Time*Condition P=0.102
Control
Prazosin
VTI (%change)
time (s)
D
P< 0.05
VTI (%change)
Control
Prazosin

FIGURE 4.

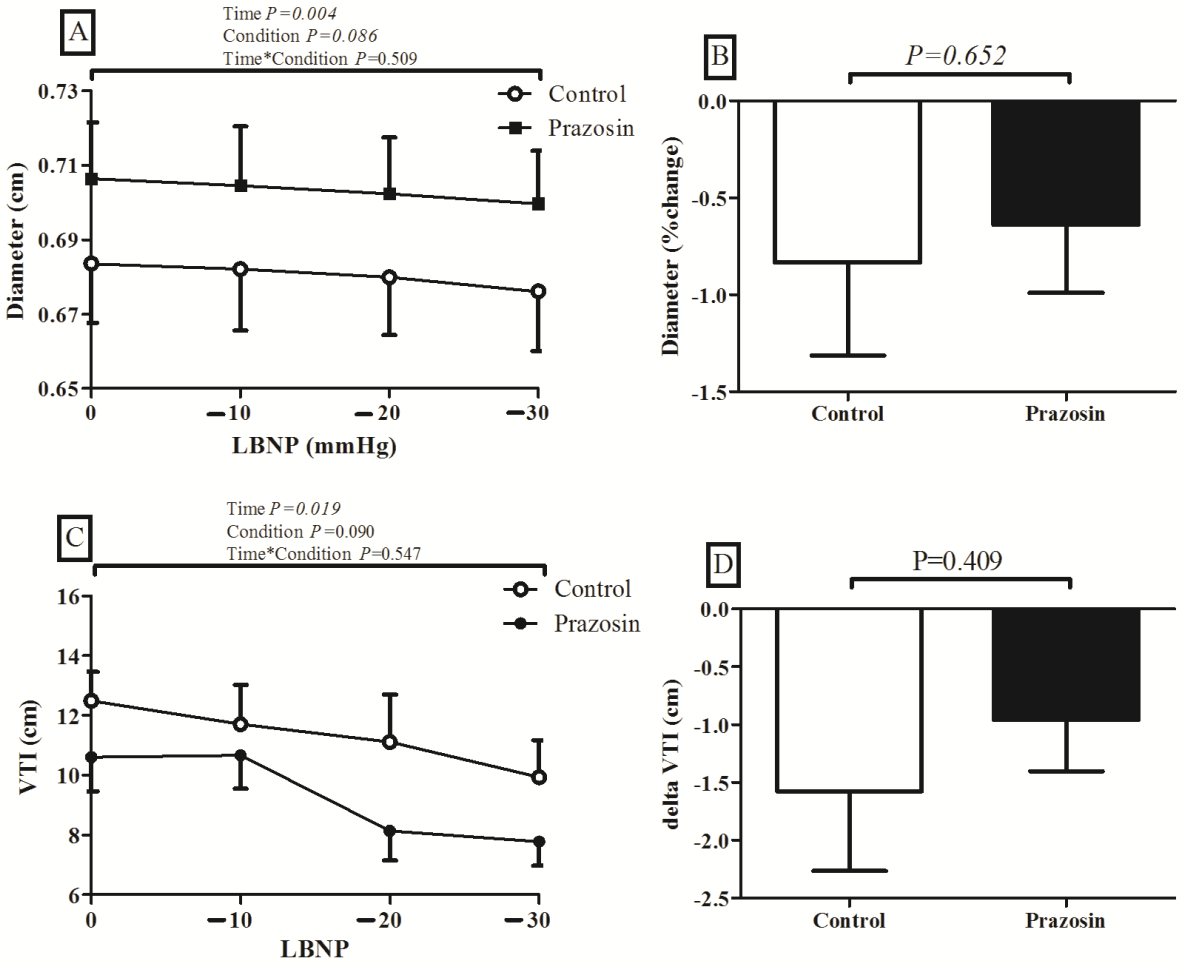
A
Time P=0.004
Condition P=0.086
Time*Condition P=0.509
Control
Prazosin
Diameter (cm)
0.73
0.71
0.69
0.67
0.65
0
10
20
30
LBNP (mmHg)
B
P=0.652
Diameter (%change)
0.0
-0.5
-1.0
-1.5
Control
Prazosin
C
Time P=0.019
Condition P=0.090
Time*Condition P=0.547
Control
Prazosin
VTI (cm)
16
14
12
10
8
6
0
10
20
30
LBNP
D
P=0.409
delta VTI (cm)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Control
Prazosin

TABLE 1 – Cold pressor test responses

Cold pressor test	1 minute CPT									2 minutes CPT						3 minutes CPT				2-way ANOVA		
	Baseline	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**10 90**	**100**	**110**	**120**	**130**	**140**	**150**	**160**	**170**	**180**	**Time**	**Trial**	**Time*Trial**
Stroke volume (ml) Control	105±15	106±17	106±16	105±17	105±17	105±19	105±20	105±21	104±20	104±20	103±20	105±21	106±21	106±21	106±20	106±20	106±20	107±19	107±19	0.450	0.296	0.450
Stroke volume (ml) Prazosin	112±14	113±15	114±14	114±15	113±16	112±17	111±16	110±15	111±17	111±16	109±17	110±17	110±17	111±17	112±17	111±17	111±18	112±18	112±17			
Cardiac Output (L/min) Control	6.2±1.3	6.6±1.2	6.8±1.3	6.6±1.4	6.7±1.6	6.8±1.8	7.0±1.6	6.9±1.6	6.7±1.7	6.6±1.5	6.3±1.4	6.3±1.5	6.3±1.4	6.3±1.5	6.1±1.6	6.3±1.5	6.3±1.4	6.3±1.5	6.3±1.4	0.200	*0.049*	*0.021*
Cardiac Output (L/min) Prazosin	6.7±0.9	7.4±1.0	7.5±1.2	7.5±1.3	7.7±1.5	7.7±1.6	7.5±1.6	7.6±1.6	7.5±1.4	7.5±1.4	7.5±1.4	7.6±1.3	7.6±1.2	7.4±1.1	7.6±1.1	7.6±1.1	7.6±1.0	7.7±0.9	7.6±1.0			
Heart Rate (bpm) Control	59±12	63±10	65±11	64±11	64±13	64±12	67±12	66±12	64±13	63±13	62±12	62±13	61±12	61±13	59±15	61±13	61±12	60±14	59±12	0.107	*0.024*	*0.001*
Heart Rate (bpm) Prazosin	62±12	69±13	68±11	68±11	71±13	71±14	70±13	71±14	70±15	70±14	71±14	72±14	72±14*	70±13	71±13*	71±12	71±13*	71±12*	70±13*			
Diastolic BP (mmHg) Control	79±8	83±6	81±7	84±9	86±8	88±8*	91±8*	92±9*	92±8	92±8	93±9*	92±9*	92±9*	92±9*	91±8*	90±8*	90±8*	90±8	89±8*	*0.000*	*0.045*	*0.031*
Diastolic BP (mmHg) Prazosin	74±9	76±9	74±9	76±9	78±9	80±9	81±10	81±9	81±9	82±8*	82±9*	82±8	83±9	80±9	81±9*	82±9*	81±9*	81±9*	80±8*			
Systolic BP (mmHg) Control	133±7	139±8	137±10	140±11	142±12	146±13	148±11*	150±12*	150±12*	150±13	151±13	150±13	151±12	151±11*	150±12*	149±11*	148±10*	148±11	148±11*	*0.000*	0.169	*0.023*
Systolic BP (mmHg) Prazosin	131±7	136±6*	135±7	136±7	138±6	140±7	141±9	140±7	140±7	140±6	141±8	141±8	141±9	139±9	140±8	141±9*	141±9*	140±9*	140±7*			
Rate pressure product - Control	7927±1754	8787±1449	8963±1654	9028±1804	9160±2048	9483±2096	9901±2047	9978±2093	9619±2114	9549±2097	9458±1931	9253±2024	9212±1982	9166±2045*	8890±2087	9077±2011	8984±1819	8883±2042	8834±1927	*0.008*	0.307	*0.014*
Rate pressure product - Prazosin	8141±1534	9380±1761	9169±1724	9284±1750	9771±1778	9953±2062	9833±2023	9960±2072	9887±2196	9858±2024	10088±2018	10158±2138	10217±2204	9705±1855	9892±1870*	9941±1667*	9981±1580*	9928±1482*	9826±1709*			
LAD velocity max Control	0.252±0.03			0.325±0.07			0.304±0.03			0.280±0.05			0.261±0.04			0.266±0.05			0.265±0.05	0.007	0.769	0.594
LAD velocity max Prazosin	0.261±0.04			0.312±0.04			0.302±0.07			0.281±0.06			0.278±0.06			0.284±0.06			0.279±0.05			
LAD velocity mean Control	0.201±0.03			0.256±0.07			0.232±0.03			0.224±0.04			0.209±0.03			0.215±0.03			0.207±0.03	0.041	0.603	0.400
LAD velocity mean Prazosin	0.199±0.03			0.25±0.02			0.226±0.03			0.228±0.05			0.231±0.04			0.231±0.04			0.228±0.04			

Hemodynamic and coronary responses during Cold pressor test (averaged per 10 second intervals). P-values refer to 2-way repeated measures ANOVA's, for within participant comparison (time), between trial comparison, and the interaction time*trial. *Symbols denote P<0.05 difference to baseline values.

TABLE 2 – Lower body negative pressure responses

Lower body negative pressure								2-way ANOVA		
	Baseline	-10		-20		-30		Time	Trial	Time*Trial
Stroke volume (ml) Control	106±13	102±12	100±12	97±12	97±13	93±14	90±14	*0.000*	0.687	0.086
Stroke volume (ml) Prazosin	106±12	103±14	99±16	96±15	93±16	91±16	85±18*			
Cardiac Output (L/min) Control	6.4±1.2	6.1±1.1	6.1±1.1	5.9±1.1	6.0±1.0	5.8±1.0	5.9±1.0	*0.642*	*0.002*	0.162
Cardiac Output (L/min) Prazosin	7.1±1.4	7.1±1.2	7.2±1.4	7.2±1.0	7.4±0.9	7.5±0.8	7.3±0.9			
Heart Rate (bpm) Control	61±12	60±12	61±13	62±13	63±13	64±14	67±14	*0.000*	*0.002*	*0.009*
Heart Rate (bpm) Prazosin	68±16	70±16	75±20	77±17*	82±17*	84±17*	89±17*			
Diastolic BP (mmHg) Control	77±9	77±9	77±9	78±9	78±9	79±9	79±10	*0.177*	0.160	0.060
Diastolic BP (mmHg) Prazosin	73±8	74±8	72±9	74±8	73±8	74±9	73±9			
Systolic BP (mmHg) Control	132±7	131±6	129±7	130±7	131±6	130±7	131±7	0.152	0.388	*0.005*
Systolic BP (mmHg) Prazosin	131±9	131±9	128±9	129±9	127±10	127±10	124±11			
Rate pressure product - Control	8048±1746	7923±1745	7929±1831	8024±1766	8192±1741	8348±1895	8708±1916	*<0.001*	*0.027*	*0.025*
Rate pressure product - Prazosin	8954±2618	9253±2537	9646±2963	9969±2491*	10479±2585*	10751±2568*	10960±2319*			
LAD VTI Control	12.1±2.7	11.1±3.6		9.9±3.4		8.7±1.3		*0.019*	0.09	0.547
LAD VTI Prazosin	9.5±0.9	9.8±1.9		8.1±2.2		7.8±1.8				
LAD velocity max Control	0.277±0.05	0.26±0.06		0.243±0.05		0.226±0.03		*0.029*	0.52	0.621
LAD velocity max Prazosin	0.25±0.06	0.249±0.03		0.226±0.04		0.225±0.04				
LAD velocity mean Control	0.21±0.03	0.19±0.03		0.203±0.04		0.187±0.03		0.496	0.973	0.177
LAD velocity mean Prazosin	0.197±0.03	0.207±0.02		0.188±0.03		0.20±0.03				

Hemodynamic and coronary responses during Lower body negative pressure test (averaged per 2 minute stages). P-values refer to 2-way repeated measures ANOVA's, for within participant comparison (time), between trial comparison, and the interaction time*trial. *Symbols denote P<0.05 difference to baseline values.