Chronic in vivo or acute in vitro resveratrol attenuates endothelium-dependent cyclooxygenase-mediated contractile signaling in hypertensive rat carotid artery

AUTHORS:

Steven G. Denniss¹, Rebecca J. Ford¹, Christopher S. Smith, Andrew J. Jeffery, and James W.E. Rush


¹ Contributed equally to this work as first authors.

AFFILIATION:

Integrative Vascular Biology Laboratory, Department of Kinesiology, Faculty of Applied Health Sciences, University of Waterloo, Waterloo, Ontario, Canada N2L3G1

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CORRESPONDING AUTHOR:

Dr. James W.E. Rush (Ph.D.)
Professor
Department of Kinesiology, Faculty of Applied Health Sciences
University of Waterloo
Waterloo, Ontario, Canada
N2L3G1
1-519-888-4567 ext. 32126
jwerush@uwaterloo.ca
ABSTRACT

Exaggerated cyclooxygenase (COX) and thromboxane-prostanoid (TP) receptor-mediated endothelium-dependent contraction can contribute to endothelial dysfunction. This study examined the effect of resveratrol (RSV) on endothelium-dependent contraction and cell-signaling in the common carotid artery (CCA) from spontaneously hypertensive (SHR) and Wistar Kyoto rats (WKY). Acetylcholine (Ach)-stimulated endothelium-dependent nitric oxide synthase (NOS)-mediated relaxation in pre-contracted SHR CCA was impaired (max.: 73±6% vs. 87±5% in WKY) (p<0.05) by competitive COX-mediated contraction. Chronic (28d) treatment in vivo (drinking water) with a ~0.075 mg•kg⁻¹•d⁻¹ RSV dose neither affected endothelium-dependent relaxation, nor endothelium-dependent contraction and associated prostaglandin (PG) production evaluated in non-precontracted NOS-blocked CCA. In contrast, a chronic ~7.5 mg•kg⁻¹•d⁻¹ RSV dose improved endothelium-dependent relaxation (94±6%), and attenuated endothelium-dependent contraction (58±4% vs. 73±5% in No RSV) and PG production (183±43 vs. 519±93 pg•ml⁻¹), in SHR CCA, while U46619-stimulated TP receptor-mediated contraction was unaffected. In separate acute in vitro experiments, 20µM RSV preincubation attenuated endothelium-dependent contraction (6±4% vs. 62±2% in No Drug) and PG production (121±15 vs. 491±93 pg•ml⁻¹), and attenuated U46619-stimulated contraction (134±5% vs. 171±4%), in non-precontracted NOS-blocked SHR CCA. Compound C, a known AMP-activated protein kinase (AMPK) inhibitor, did not prevent the RSV attenuating effect on Ach- and U46619-stimulated contraction, but did prevent the RSV attenuating effect on PG production (414±58 pg•ml⁻¹). These data demonstrate that RSV can attenuate endothelium-dependent contraction both by suppressing arterial wall PG production, which may be partially
mediated by AMPK, and by TP receptor hypo-responsiveness, which does not appear to be mediated by AMPK.

**KEYWORDS:**

Endothelium-dependent contraction; resveratrol; cyclooxygenase; prostaglandin; thromboxane-prostanoid receptor; AMP-activated protein kinase
NEW & NOTEWORTHY

These experiments were designed to investigate the under-studied role of RSV in affecting COX and TP receptor-mediated endothelium-dependent contraction that can contribute to endothelial dysfunction. The data demonstrate for the first time that both chronic in vivo RSV treatment and acute in vitro RSV exposure can attenuate endothelium-dependent contraction, and that this attenuating effect can occur in the arterial wall not only by suppressing PG production via AMPK, but also by inhibiting TP receptor activity.
ABBREVIATIONS:

Ach - Acetylcholine
AICAR - 5-Aminoimidazole-4-Carboxamide
AMPK - AMP-activated protein kinase
AUC - Area under the curve
CC - Compound C
CCA - Common carotid artery
COX - Cyclooxygenase
EC50 - Half-maximal effective concentration
High-RSV - High-Dose Resveratrol Treatment
Indo - Indomethacin
ʟ-NAME - \(N^\omega\)-nitro-ʟ-arginine methyl ester
Max Amp - Maximum amplitude
Mod-RSV - Moderate-Dose Resveratrol Treatment
No-RSV - No Resveratrol Treatment
NOS - Nitric oxide synthase
PG - Prostaglandin
RSV - Resveratrol
SHR - Spontaneously Hypertensive rat
TP - Thromboxane-Prostanoid
WKY - Wistar Kyoto rat
INTRODUCTION

Endothelial dysfunction can be caused by under-active signaling of endothelium-derived relaxing factors, including nitric oxide, prostaglandin (PG) species, and endothelium-derived hyperpolarizing factors (7), and/or by over-active signaling of endothelium-derived contracting factors (4, 7). Primary candidates for endothelium-derived contracting factors include cyclooxygenase (COX)-derived PG species (4, 5), which induce vascular smooth muscle contraction via thromboxane-prostanoid (TP) receptor activation (6). Many mechanisms of endothelium-dependent vasocontractile activity and cell-signalling have been elucidated using the archetypal spontaneously hypertensive rat (SHR) and normotensive Wistar Kyoto rat (WKY) conduit artery model (4, 6, 7, 26, 30). In the aorta (8, 11, 30) and common carotid artery (CCA) (2, 3) from these animals, competitive endothelium-dependent COX- and TP receptor-mediated vasocontraction is a major cause of impaired endothelium-dependent vasorelaxation.

Resveratrol (RSV; 3,4,5’-trihydroxystilbene) is a naturally occurring polyphenol found in red wine, and has been associated with protection against cardiovascular disease (17). Research investigating potential mechanisms of action of RSV on endothelial dysfunction in precontracted arterial segments from animal models of cardiovascular disease risk has focussed primarily on whether RSV mitigates under-active nitric oxide-mediated vasorelaxation (16, 21, 23). Such investigations have found that RSV may act via multiple acute and chronic mechanisms to protect against endothelial dysfunction by promoting an increase in endothelial nitric oxide synthase (NOS)-derived nitric oxide production and/or a decrease in oxidative stress-mediated nitric oxide destruction. Previous studies have also found that acute in vitro RSV exposure (18)
can attenuate endothelium-dependent contraction in arteries from aged diabetic rats and that chronic in vivo treatment with a red wine polyphenol extract (12) has a similar effect on arteries from angiotensin II-infused rats. Together with evidence that COX is known to be a target of RSV (17, 21), these observations suggest a potential role for RSV in suppression of vasoconstriction. However, the effects of either acute or chronic RSV on endothelium-dependent vasocontractile activity and cell-signaling have yet to be investigated in the SHR–WKY model. Furthermore, AMP-activated protein kinase (AMPK) is also known to be a target of RSV (17, 21), and acute AMPK activation has been found to attenuate endothelium-dependent contraction in SHR and WKY aorta (8). However, the possible role of AMPK in mediating the effects of RSV on endothelium-dependent vasocontractile activity and cell-signaling has yet to be investigated in any model of cardiovascular disease risk.

Accordingly, the main objectives of the current study were to determine the effects of chronic in vivo RSV treatment, or acute in vitro RSV exposure, on endothelium-dependent COX- and TP receptor-mediated contractile activity and cell-signaling in SHR and WKY CCA. It was hypothesized that chronic RSV treatment would attenuate endothelial dysfunction caused by over-active endothelium-dependent contraction in SHR CCA (2) by a mechanism involving the suppression of PG production (3). It was further hypothesized that the acute effects of RSV on endothelium-dependent contractile activity and cell-signaling would be similar to those found following chronic treatment, and would depend in part on AMPK (8).
MATERIALS AND METHODS

Reagents and Drugs

Unless otherwise stated, all reagents and drugs were purchased from Sigma-Aldrich (St Louis, MO) or BioShop Canada (Burlington, ON, Canada), and all drugs used in vitro were first prepared as 1000x stock solutions in ultra-pure water.

Animals and Arterial Segment

The isolated CCA of 20-24 week old male SHR and WKY was used as a bioassay of endothelium-dependent vasomotor function in all experiments. SHR and WKY were purchased from Harlan Laboratories (Indianapolis IN), received at 12 weeks of age, and housed four per cage in a temperature-controlled facility (21 ± 1°C) on a reversed 12:12 hour light:dark cycle, having free access to standard laboratory chow and tap water. All procedures involving animals were approved by the University of Waterloo Animal Care Committee and were in accordance with the Guidelines of the Canadian Council on Animal Care and the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals.

A 50-65 mg/kg body weight intra-peritoneal injection of sodium pentobarbital (Bimeda-MYC, Cambridge, ON, Canada) was used to anesthetize each animal prior to tissue extraction.

Chronic In Vivo RSV Treatment: Experimental Design and Testing
The chronic in vivo RSV treatment was performed in a similar way as previously described (23). Briefly, the treatment consisted of a 28-day *ad libitum* oral supplementation in the drinking water with either: vehicle with no RSV (No-RSV (Control)), consisting of tap water with 1g•100 ml⁻¹ low viscosity carboxymethyl cellulose vehicle only (MP Biomedicals, Solon OH); a ‘moderate’ dose of RSV (Mod-RSV), consisting of tap water-carboxymethyl cellulose with 0.64 mg•l⁻¹ *trans* RSV (Toronto Research Chemicals, Toronto ON Canada), targeted to achieve an ~0.075 mg•kg⁻¹•day⁻¹ dose of RSV, designed to mimic ‘moderate’ daily red wine consumption in humans (23); or a ‘high’ dose of RSV (High-RSV), consisting of tap water-carboxymethyl cellulose with 64 mg•l⁻¹ *trans* RSV, targeted to achieve an ~7.5 mg•kg⁻¹•day⁻¹ dose of RSV, designed to result in consumption at ~100x the Mod-RSV dose.

At 20 weeks of age, WKY and SHR were weighed, moved from group housing to individual housing within the same facility, and randomized into one of the three drinking water treatment groups: No-RSV, Mod-RSV, or High-RSV. Each individually-housed animal had free access to standard laboratory chow and assigned water bottle. Water bottles were wrapped in tinfoil to prevent photo-degradation of RSV. Water bottles were changed daily at which time the amount of water and food consumed by each animal was recorded.

Following the 28-day treatment, animals were anesthetized and mean arterial blood pressure was assessed using a Millar catheter-tip pressure transducer inserted into the left CCA as previously described (2), after which CCA tissue was excised and prepared for in vitro assessment of vasomotor functions and PG production as described below (or protein expression, as described previously (2, 8)).

*Acute In Vitro RSV Exposure: Experimental Design and Testing*
The acute in vitro RSV exposure experiments were performed in a similar way as previously described (2, 3, 8). CCA rings excised from anesthetized 20-24 week old group-housed SHR and WKY were mounted in tissue baths and randomized to be pre-incubated for 30 min in buffer with or without trans RSV (RSV-20µM; stock 20 mM RSV dissolved in N2-purged ethanol) prior to the assessment of vasomotor functions and PG production, as described below. The standard acute RSV protocol was chosen after initial RSV dose-response experiments demonstrated that RSV-20µM was as effective as higher in-bath concentrations (up to 100 µM RSV) at attenuating endothelium-dependent vasocontractile activity (see RESULTS and Fig. 4).

**Tissue Excision and Preparations**

Tissue excision and preparation was performed in a similar way as previously described (2, 3, 8). In brief, for both chronic RSV treatment experiments and acute RSV exposure experiments, anesthetized animals were killed by removal of the heart. Intact CCA tissue (right and/or left, depending on whether blood pressure measures were performed; arteries that had been catheterized to perform blood pressure assessments were not used for further functional or biochemical analyses) was carefully excised and cleaned of surrounding connective tissue in 4°C buffer solution containing (in mM): 131.5 NaCl, 13.5 NaHCO₃, 11.2 glucose, 5.0 KCl, 2.5 CaCl₂, 1.2 NaH₂PO₄, 1.2 MgCl₂, and 0.025 EDTA, which was prepared fresh daily in ultrapure water at pH 7.40.

**Assessment of Agonist-Stimulated CCA Vasomotor Function**

Vasomotor function of CCA rings was assessed in a similar way as previously described (2, 3, 8). Briefly, rings of 2.0 mm axial length were prepared using a dissecting microscope (Zeiss;
VWR, Mississauga, ON, Canada). Rings were mounted on a myography apparatus and equilibrated at optimal length for stimulated isometric tension production in a 37°C tissue bath containing 5 ml of 95% O2-5% CO2-gassed buffer solution.

Rings were then stimulated to contract twice with 60 mM KCl and washed with buffer in between. The maximum amplitude of the contractile response to the second exposure to 60 mM KCl was used as a reference contraction for each ring.

Next, rings were randomly assigned to a 30 min preincubation in either buffer without any drug(s) (No drug) or buffer with relevant enzyme or protein inhibitor(s) or activator(s).

In particular, No-RSV, Mod-RSV, and High-RSV rings isolated from the chronic in vivo RSV-treated SHR and WKY were preincubated with either: No Drug; the non-selective COX-1/-2 inhibitor indomethacin (Indo; 10^{-5} M; prepared as a 1000x stock solution in dimethyl sulfoxide; (3)); or the non-selective NOS inhibitor N\textsuperscript{ω}-nitro-L-arginine methyl ester (L-NAME; 10^{-4} M; (3)).

Rings isolated from SHR and WKY used for the acute in vitro RSV exposure experiments were preincubated with either: L-NAME; L-NAME and RSV (L-NAME + RSV-20\mu M (or -1\mu M, -3\mu M, -10\mu M, or -100\mu M)); L-NAME and the known AMPK activator 5-Aminoimidazole-4-Carboxamide (AICAR; 10^{-4.5} M; (8); Toronto Research Chemical, Toronto ON Canada) (L-NAME + AICAR); L-NAME and the known selective AMPK inhibitor Compound C (CC; 10^{-4.5} M; (8); Calbiochem) (L-NAME + CC); L-NAME and AICAR and CC (L-NAME + AICAR + CC; (8)); or L-NAME and RSV-20\mu M and CC (L-NAME + RSV-20\mu M + CC).

Following the preincubation period, vasomotor function was assessed.

In particular, agonist-stimulated endothelium-dependent vasorelaxation was assessed in rings precontracted with the \(\alpha_1\)-adrenergic receptor agonist phenylephrine (10^{-6} M; (2)) and then
exposed to increasing concentrations of the muscarinic receptor agonist acetylcholine (Ach; -9.0 to -4.0 Log M; (2)).

Endothelium-dependent vasocontraction in response to cumulative Ach was assessed in rings starting from a quiescent (i.e. non-pre-contracted) state and always preincubated with L-NAME to inhibit competitive Ach-stimulated nitric oxide-mediated signaling activity, thus optimizing the specific evaluation of endothelium-dependent contractile activity and cell-signaling (2, 3, 8).

The vasocontraction response to cumulative exposure to the TP receptor agonist U46619 (-9.0 to -6.0 Log M; Cayman Chemical, Ann Arbor, MI; 1000x stock dissolved in N2-purged ethanol) was assessed in rings starting from a quiescent state.

**Assessment of Ach-Stimulated CCA PG Production**

As previously described (3, 11), PG production from rings was assessed by collecting the buffer from the tissue baths immediately following peak contraction to -4.0 Log M Ach in the Ach-stimulated vasocontractile response. Buffer was flash-frozen, stored at -80C, and later used in a competitive enzyme-linked immunosorbent assay (Cayman Chemical; according to the manufacturer’s instructions) to determine the concentration of 6-keto PGF\(_1\alpha\), a stable product of the non-enzymatic hydrolysis of unstable PGI\(_2\). The COX- and PGI synthase-derived endoperoxide derivative PGI\(_2\) was chosen for analysis because previous studies (26) including those by the authors (3, 11) have found that PGI\(_2\) is the main PG produced from arterial rings from SHR and WKY in response to the endothelial agonist Ach, and because, while traditionally known as a vasodilator, PGI\(_2\) in arteries of the SHR-WKY model elicits a TP receptor-mediated vasocontractile response because of dysfunctional IP receptors (9, 10).
Data Analysis and Statistics

Data are expressed as means ± SEM. Details of data collection and curve-fitting analysis procedures have been previously described in detail (2). Curve-fitting analysis was performed using GraphPad Prism 4 (La Jolla, CA) to produce Maximum Amplitude (Max Amp), half-maximal effective concentration (EC$_{50}$), and Area Under the Curve (AUC) parameters, which were then used for statistical comparisons. Statistical analyses were performed using GraphPad Prism 4 (La Jolla, CA). Two-tailed, independent-sample Student’s t-tests were used for single-point comparisons, and one- or two-way ANOVA were used for multiple within and/or between group comparisons with Bonferroni post-hoc tests used when the main effects from the ANOVA were statistically significant. Differences in comparisons were considered statistically significant if $P$ values were <0.050.

RESULTS

Effects of Chronic In Vivo RSV Treatment

Animal Characteristics and Estimation of RSV Consumption

Before RSV treatment, body weights of SHR were ~5-10% greater than those of WKY (Table 1), which is not unexpected (2). All animals gained weight over the treatment period, and there was no effect of the treatments on weight gain in either strain (Table 1). Based on measurement of the disappearance of food and water from their containers, SHR consumed ~5-
15% more food and ~25-35% more water than WKY over the treatment period (Table 1), an
effect that has been documented previously (13). It cannot be known for sure whether these
increases were solely due to an increase in actual intake, or, for example, due to increased
spillage (13). There was no effect of the treatments on food or water consumption in either strain
(Table 1).

Based on the daily water consumption amounts measured, the estimated RSV consumed
by Mod-RSV and High-RSV groups closely approximated the targeted doses of ~0.075 mg•kg⁻¹•day⁻¹, and ~7.500 mg•kg⁻¹•day⁻¹, respectively (Table 1). The estimated amount of RSV
consumed relative to body weight in both the Mod-RSV and High-RSV treatment conditions was
~25-30% higher in SHR than in WKY because of the measured increase in water consumption
by SHR (Table 1).

Mean arterial pressure was elevated in SHR compared to WKY (Table 1). Compared to
No-RSV, neither Mod-RSV nor High-RSV significantly affected mean arterial pressure in WKY
or SHR.

High-RSV Reverses Impaired Endothelium-Dependent Relaxation Caused by COX-Mediated
Contraction in SHR CCA

Ach elicited a similar, robust dose-dependent relaxation response in WKY No Drug No-
RSV rings (n=8), No Drug Mod-RSV rings (n=8), and No Drug High-RSV rings (n=8) (Fig. 1,
left panel; and Table 2). In SHR No Drug No-RSV rings (n=9) (Fig. 1, right panel; and Table 2),
Ach-stimulated relaxation was blunted compared to WKY (SHR vs. WKY Max Amp and AUC;
p<0.001), while the sensitivity to Ach was greater (SHR vs. WKY EC50; p<0.001). Between -9.0
and -6.0 Log M Ach, SHR rings relaxed to a greater extent relative to WKY rings. Between -6.0
and -4.0 Log M, in contrast to the effect in WKY rings, there was no additional relaxation in SHR rings, but rather a tendency for ‘recontraction’ (Fig. 1, left and right panels), which has been demonstrated previously (2, 3, 7).

Mod-RSV had no effect on Ach-stimulated relaxation in No Drug SHR rings (n=8) (Fig. 1, right panel; and Table 2), whereas High-RSV reversed the impaired relaxation to higher Ach doses in SHR rings (n=8) (Fig. 1, right panel; and Table 2) resulting in a relaxation amplitude between -6.0 and -4.0 Log M Ach that was as robust as WKY (Fig. 1, left and right panels).

Indo reversed the impaired relaxation to higher Ach doses in No-RSV SHR rings (n=5) resulting in a response that was similar to High-RSV SHR rings and to WKY rings (Fig. 1, left and right panels; and Table 1), thus confirming, as has been previously demonstrated (2, 3), that the impaired Ach-stimulated endothelium-dependent relaxation found in SHR CCA was caused by COX-mediated endothelium-dependent contractile cell-signaling activity.

The relaxation response to cumulative sodium nitroprusside, a nitric oxide donor commonly used to test the sensitivity of vascular smooth muscle to endothelium-independent nitric oxide-mediated relaxation (2), was no different between strains or within a strain across treatment conditions (data not shown).

High-RSV Attenuates Endothelium-Dependent Contraction in SHR CCA

Ach elicited a contractile response in l-NAME No-RSV WKY rings (n=5), which was unaffected by Mod-RSV (n=4) or High-RSV (n=5) (Fig. 2A, left panel; and Table 2).

Similar to previous observations (3), Ach elicited a contractile response in l-NAME No-RSV SHR rings (n=7) that was >2.5-fold more robust than in WKY (SHR vs. WKY Max Amp; p<0.001) (Fig. 2A, right panel; and Table 2). Mod-RSV had no effect on the Ach-stimulated
contraction in SHR rings (n=5), whereas High-RSV (n=5) resulted in a ~20% attenuation of Max Amp (Fig. 2A, right panel; and Table 2).

High-RSV Attenuates Stimulated PG Production from SHR CCA

In response to cumulative Ach depicted in Fig. 2A, the production of 6-keto PGF1α from l-NAME No-RSV SHR rings (519±93 pg•ml⁻¹; Fig. 2B, right panel) was ~3.5-fold higher compared to that from No-RSV WKY rings (144±34 pg•ml⁻¹; Fig. 2B, left panel) (p<0.01). PG production in WKY rings was unaffected by Mod-RSV (104±14 pg•ml⁻¹) or High-RSV (142±28 pg•ml⁻¹) (Fig. 2B, left panel). PG production was not significantly reduced by Mod-RSV in SHR rings (299±51 pg•ml⁻¹; Fig. 2B, right panel), whereas High-RSV resulted in a significant ~65% attenuation of PG production (183±43 pg•ml⁻¹; Fig. 2B, right panel) (p=0.02) such that it was similar to that produced from WKY rings.

TP Receptor-Mediated Contraction Unaffected by High-RSV

U46619 elicited a comparable contractile response in No Drug No-RSV SHR rings (n=5) and No Drug No-RSV WKY rings (n=3), and in No Drug High-RSV SHR rings (n=5) and No Drug High-RSV WKY rings (n=5) (Fig. 3, right and left panel, respectively; and Table 2).

Effects of Acute In Vitro RSV Exposure
For simplicity, only the SHR data corresponding to the acute in-bath RSV exposure experiments are presented herein given that: the pattern of the relative differences in magnitudes of the Ach- and U46619-stimulated vasomotor dose-responses across the in-bath drug pre-incubation conditions was found to be similar in SHR and WKY CCA; and the absolute magnitudes of the Ach-stimulated vasomotor dose-responses were significantly greater in SHR CCA, and thus better illustrated.

Acute RSV Causes a Dose-Dependent Attenuation of Maximal Endothelium-Dependent CCA Contraction

Acute RSV preincubation caused a dose-dependent attenuation of the contractile response to -4.0 Log M Ach in SHR rings, plateauing at RSV-20µM, which resulted in a ~90% attenuation of the Ach-stimulated contraction compared to the response in the absence of RSV (Max Amp: 65.0±1.4% with l-NAME vs. 7.5±2.4% with l-NAME+RSV-20µM; n=4; p<0.001) (Fig. 4).

Based on these initial observations, 20 µM RSV was chosen as the in-bath dosage used for all remaining acute in vitro experiments.

RSV-20µM or AICARNearly Eliminate Endothelium-Dependent CCA Contraction

RSV-20µM (l-NAME+RSV-20µM (n=6)) attenuated the Ach-stimulated contraction response by ~90% compared to its control (l-NAME (n=6)) (Fig. 5A; and Table 3).

AICAR (l-NAME+AICAR (n=4)) attenuated Ach-stimulated contraction to a similar extent as RSV-20µM (Fig. 5A; and Table 3).
CC (l-NAME+CC; n=3)) had no effect on Ach-stimulated contraction, whereas AICAR+CC (l-NAME+AICAR+CC; n=4) completely reversed the attenuating effect of AICAR (Fig. 5A; and Table 3), thereby suggesting an AMPK specificity of the observed functional effect of AICAR.

Noteworthy, in isolated l-NAME-preincubated SHR and WKY aortic rings, the authors confirmed a similar pattern and magnitude of effect of AICAR, CC, and AICAR+CC on the Ach-stimulated contractile response as in Fig. 5A and Table 3 (data not shown), and confirmed AMPK activation with AICAR, and inhibition with AICAR+CC, by western blotting, as previously described (8), for phospho(Thr^{172})-AMPK and phospho(Ser^{79})-acetyl-CoA carboxylase protein expression (~3-4 fold increase in both phospho(Thr^{172})-AMPK and phospho(Ser^{79})-acetyl-CoA carboxylase) (in snap-frozen SHR and WKY aortic rings following contraction stimulated by cumulative Ach; data not shown).

RSV-20µM and CC combined (l-NAME+RSV-20µM+CC; n=5) did not even partially restore Ach-stimulated contraction (Fig. 5A; and Table 3), thereby suggesting that the attenuating effect of RSV on endothelium-dependent contractile activity and cell-signaling was not dependent on AMPK.

RSV-20uM or AICAR Markedly Attenuate Stimulated CCA PG Production

In response to cumulative Ach depicted in Fig. 5A, PG production from SHR CCA rings was attenuated ~75% by RSV-20µM (l-NAME+RSV-20µM; 121±15 pg•ml^{-1}) compared to its control (l-NAME; 491±93 pg•ml^{-1}) (p<0.001), while AICAR attenuated PG production by ~55% (l-NAME+AICAR; 242±39 pg•ml^{-1}) (p=0.04) (Fig. 5B). PG production was not significantly affected by CC (l-NAME+CC; 713±65 pg•ml^{-1}) or AICAR+CC (l-NAME+AICAR+CC;
695±72 pg•ml⁻¹) (Fig. 5B), thereby suggesting an AMPK specificity of the observed biochemical
effect of AICAR.

Noteworthy, in isolated l-NAME-preincubated SHR and WKY aortic rings, the authors
confirmed a similar pattern and magnitude of effect of AICAR, CC, and AICAR+CC on
stimulated PG production as in Fig. 5A and Table 3 (data not shown).

PG production was not found to be attenuated by RSV-20µM and CC combined (l-
NAME+RSV-20µM+CC; 414±58 pg•ml⁻¹) (Fig. 5B), thereby suggesting that the attenuating
effect of RSV on stimulated PG production may have been mediated by a RSV-dependent
increase in CCA AMPK cell-signaling activity.

**RSV-20µM but not AICAR Partially Attenuates TP Receptor-Mediated CCA Contraction**

In the same SHR CCA rings as represented in Fig. 5A and 5B (following exposure to
cumulative Ach, rings were washed thoroughly to re-establish a quiescent baseline), RSV-20µM
(l-NAME+RSV-20µM) attenuated maximal contraction to U46619 by ~20% compared to its
control (l-NAME).

U46619-stimulated contraction was unaffected by AICAR (l-NAME+AICAR), CC (l-
NAME+CC), or AICAR and CC combined (l-NAME+AICAR+CC) (Fig. 6; and Table 3).

Noteworthy, in isolated l-NAME-preincubated SHR and WKY aortic rings, the authors
confirmed a similar lack of effect of AICAR, CC, and AICAR+CC on PGH₂-stimulated
contraction as on U46619-stimulated contraction in Fig. 6; and Table 3 (data not shown)

RSV-20µM and CC combined (l-NAME+RSV-20µM+CC) attenuated maximal
contraction to U46619 by a similar magnitude as RSV-20µM (l-NAME+RSV-20µM) (Fig. 6;
and Table 3), thereby suggesting that the attenuating effect of RSV on TP receptor-mediated contractile activity was not dependent on AMPK.

**DISCUSSION**

The major findings of the current study are that: 1) chronic in vivo high-dose RSV treatment reversed the impaired Ach-stimulated endothelium-dependent relaxation in SHR CCA; 2) both high-dose chronic RSV treatment, and acute in vitro RSV-20µM exposure, attenuated Ach-stimulated endothelium-dependent contractile activity and associated PG production in CCA; 3) RSV-20µM blunted maximal U46619-stimulated TP receptor-mediated CCA contractile activity; and 4) AMPK inhibition did not affect the ability of RSV-20µM to attenuate either U46619-stimulated contraction, or Ach-stimulated endothelium-dependent contraction, but did eliminate the ability of RSV-20µM to attenuate the associated Ach-stimulated PG production. These findings further elucidate the COX-, PG-, and TP receptor-mediated mechanisms through which RSV may act to elicit its protective effects against endothelial dysfunction.

The findings of the current study confirm and extend previous findings regarding the chronic and acute doses of RSV necessary to provoke possible effects on endothelium-dependent vasoconstriction. This was evident in the general findings that the high (100 times moderate dose), but not the moderate (dose mimicking body weight normalized RSV ingestion resulting from moderate red wine consumption in humans; (23)) chronic RSV protocol significantly attenuated COX- and TP receptor-mediated endothelium-dependent vasoconstriction and associated PG production. This is consistent with the results of limited past works examining the effect of chronic red wine polyphenol treatment in aorta of AII-infused rats (12) and streptozotocin-induced diabetic mice (24). A concentration of ~1-5 µM has been found to be
achievable in vivo in the plasma and vasculature following chronic RSV treatment (1, 28). Matsumoto et al found that acute preincubation with 5 µM RSV was able to partially attenuate endothelium-dependent contraction in non-precontracted NOS-blocked mesenteric arterial rings from aged type-II diabetic rats (18). The current study confirms this finding in the SHR-WKY conduit artery model, and further demonstrates the acute in vitro RSV dose-dependent attenuation of endothelium-dependent contraction peaking at 20 µM RSV.

The current data also offers new insight into the cell-signaling mechanism by which RSV may act to attenuate endothelium-dependent vasoconstriction. Consistent with the pioneering work of Vanhoutte et al in SHR and WKY aorta (4, 7, 27), the authors have previously demonstrated that COX and the TP receptor are exclusively mediating Ach-stimulated endothelium-dependent contraction found in non-precontracted NOS-blocked SHR and WKY CCA (2, 3); that COX-1, and not COX-2, appears to be the isoform responsible for such CCA contraction (2, 3); and that PGI₂, and not TXA₂, is the dominant PG produced from the CCA wall upon Ach stimulation (3). Since neither COX-1 protein expression (data not shown) nor TP receptor-mediated contraction were found to be affected by the chronic high-dose RSV treatment, the attenuating effect of RSV on endothelium-dependent contraction appears to have been caused solely by suppression of CCA PG production. Given that RSV has been found to inhibit COX-1 activity in other tissues (17, 21), it appears most likely that either direct or indirect inhibition of vascular wall COX-1 activity mediated the PG production-suppressing effect found herein in response to both the chronic high-dose RSV treatment and the acute RSV-20µM exposure.

It appears that RSV is capable of attenuating endothelium-dependent contraction in the arterial wall not just by inhibiting COX-derived PG production, as demonstrated in the acute
RSV-20µM experiments herein and in previous studies (24), but also by inhibiting TP receptor-mediated contractile cell-signaling activity, as demonstrated herein by acute RSV-20µM partially attenuating TP receptor sensitivity. This new insight is consistent with past work demonstrating that RSV can attenuate TP receptor-mediated cell-signaling activity in platelets (31).

Two lines of evidence from previous studies led us to investigate the potential role of AMPK in mediating the effects of RSV observed in the current study. Firstly, endothelium-dependent vasocontractile activity and cell-signalling have been found to be attenuated by AICAR, presumably through AMPK activation, in non-precontracted NOS-blocked obese type-II diabetic rat mesenteric arteries (19) and in SHR aorta (8). Secondly, AMPK has been found to be a molecular target of RSV (17, 21). Thus, the current study investigated whether the effects of RSV-20µM on endothelium-dependent contraction, PG production, and TP receptor-mediated vasocontractile activity observed herein were affected by AICAR, and/or by CC, a presumed AMPK inhibitor (8). In consideration of potential issues relating to the efficacy of AICAR as an AMPK activator and CC as an AMPK inhibitor at different concentrations in different tissue and experimental preparations (22), the acute experiments herein included sets of molecular controls (phospho-AMPK and phospho-acetyl-CoA carboxylase; data not shown) which acted as indicators of the efficacy of AICAR and CC in the SHR-WKY conduit artery model at the concentrations used. The data herein both confirm the attenuating effects of AMPK activation on endothelium-dependent vasocontraction in SHR and WKY CCA, and offer additional mechanistic insight.

Indeed, in the current study, the effect of AICAR on endothelium-dependent vasocontractile activity did not appear to be mediated by attenuating TP receptor responsiveness, but rather by PG production-suppression only. Given that TP receptor responsiveness was
attenuated by RSV-20µM, it appears that AMPK does not mediate the attenuating effect of RSV on TP receptor vasocontractile responsiveness in the SHR-WKY conduit artery model. This interpretation is strengthened by the fact that CC was found not to be able to reverse the attenuating effect of RSV-20µM on TP receptor-mediated vasocontraction. Similarly, it also appears that AMPK does not mediate the attenuating effect of RSV on endothelium-dependent contraction in the SHR-WKY conduit artery model. Indeed, although acute preincubation with either RSV-20µM or AICAR resulted in similar attenuating effects on endothelium-dependent vasocontraction, CC was found not to be able to reverse the attenuating effect of RSV-20µM on endothelium-dependent vasocontraction.

However, in curious contrast, given that acute RSV-20µM or AICAR preincubation resulted in similar PG production-suppressing effects, and CC was found to reverse the PG production-suppressing effect of RSV-20µM, it appears from the data herein that AMPK may mediate the attenuating effect of RSV on PG production associated with the endothelium-dependent contractile activity, at least in the SHR-WKY conduit artery model.

Insomuch as we (3) and others (4, 5) have previously found that specific COX-1 inhibition, but not preferential COX-2 inhibition, completely abolishes both Ach-stimulated endothelium-dependent contraction and PG production in the SHR-WKY conduit artery model, the data herein suggest that AMPK may inhibit COX-1 activity, and that RSV, instead of acting directly to inhibit COX-1 activity (17, 21), may act indirectly through AMPK to suppress Ach-stimulated PG production. However, the authors are unaware of previous work demonstrating that AMPK can affect COX-1 activity or expression, whereas past works have demonstrated that AMPK activation can result in the acute suppression of COX-2 expression (14, 15). To reconcile the data herein with the currently available evidence, it appears feasible to suggest for further
investigation that either: COX-2 may somehow actually play a role in the Ach-stimulated PG production in SHR and WKY conduit arteries, as it does in other arteries and/or arterioles from other animal models ((4, 5) (18, 19)); or that AMPK is able to affect COX-1 activity.

Also noteworthy is that CC was found to reverse the PG production-suppressing effect of RSV-20µM, but was not found to reverse the associated attenuation of endothelium-dependent vasocontraction, or to affect TP receptor responsiveness. This suggests that COX-derived PG production from the endothelium in response to agonist stimulation may not be an exclusive causative factor in the activation of endothelium-dependent vasocontractile activity. Indeed, while COX-derived PG species have been identified as primary candidates for endothelium-derived contracting factors (4), other factors including reactive oxygen species, appear to play either a direct, or indirect, role in the endothelium-dependent vasocontractile cell-signaling axis in both SHR and WKY and other models (26). As such, the possibility that the observed dissociation observed between agonist-stimulated PG production and endothelium-dependent vasocontractile activity might be explained by other properties of RSV, including its ability to act as an anti-oxidant (17), should be investigated in future studies.

Future investigation into whether and how a change in endothelium and/or vascular smooth muscle intracellular calcium concentration may play a role in the effect of RSV on agonist-stimulated PG production, TP receptor responsiveness, and endothelium-dependent vasoconstriction is also warranted, given that altered endothelial intracellular calcium has been found to precede PG production upon agonist stimulation in the SHR-WKY conduit artery model (19, 25) and that RSV has been found to alter both endothelial and VSM cell calcium handling (20). Consistent with this rationale, RSV has been found to attenuate rat urinary bladder calcium influx, PG production, and contraction in response to agonist stimulation (29).
Conclusion

It is concluded from this study that acute in vitro exposure to 20 µM RSV, or 28 day treatment with a ~7.5 mg•kg⁻¹•day⁻¹ RSV dose, can have a nitric oxide-independent effect on endothelial function by attenuating endothelium-dependent vasocontractile activity and related COX-, PG-, and TP receptor-mediated cell-signaling. The acute cell-signaling mechanisms by which RSV can exert this effect involve both the suppression of arterial wall PG production, which appears to be mediated at least in part by AMPK, and by TP receptor hypo-responsiveness, which appears to not be mediated by AMPK.
ACKNOWLEDGEMENTS

The authors would like to thank Andrew S. Levy and Kristina K. Durham for their technical assistance.

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CONFLICTS OF INTEREST

None.
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8. **Ford RJ, Rush JWE.** Endothelium-dependent vasorelaxation to the AMPK activator AICAR is enhanced in aorta from hypertensive rats and is NO and EDCF dependent. *Am J Physiol Heart Circ Physiol* 300: H64–75, 2011.


FIGURE LEGENDS

**Fig. 1.** Vasorelaxation stimulated by cumulative acetylcholine (Ach) in phenylephrine precontracted common carotid artery rings (2 mm axial length; n = 5-9 per condition) excised from 24 week old Wistar Kyoto rats (WKY) and Spontaneously Hypertensive rats (SHR) administered either no resveratrol (RSV) (No-RSV), a moderate RSV dose (Mod-RSV), or a high (100x) RSV dose (High-RSV) in the drinking water for 28 days. Mounted arterial rings were pre-incubated with either No Drug or the non-selective cyclooxygenase inhibitor indomethacin (Indo; 10^{-5} M). Data represent means ± S.E.M. * p<0.05 denotes a significant difference among curve-fit parameters within strain between treatment conditions. See Table 2 and RESULTS for details.

**Fig. 2.** Panel A depicts the vasocontraction (expressed relative to a previous KCl reference contraction) stimulated by cumulative acetylcholine (Ach) in non-precontracted, N^\text{\textdegree}-nitro-L-arginine methyl ester (L-NAME; 100 μM) pre-incubated common carotid artery rings (2 mm axial length; n=4-7 per condition) excised from 24 week old Wistar Kyoto rats (WKY; left) and
Spontaneously Hypertensive rats (SHR; right) administered either no resveratrol (RSV) (No-RSV), a moderate RSV dose (Mod-RSV), or a high (100x) RSV dose (High-RSV) in the drinking water for 28 days. Panel B depicts the concentration of 6-keto prostaglandin (PG) F1α (a stable product of PGI2) in the bathing buffer surrounding WKY and SHR No-RSV, Mod-RSV, and High-RSV arterial rings following exposure to cumulative Ach in Panel A. * p<0.05 in Panel A denotes a significant difference among curve-fit parameters within strain between treatment conditions. * p<0.05 in Panel B denotes a significant difference within strain between treatment conditions. See Table 2 and RESULTS for details.

**Fig. 3.** Vasocontraction stimulated by cumulative U46619 (a thromboxane-prostanoid receptor agonist) in non-precontracted common carotid artery rings (2 mm axial length; n = 3-5 per condition) excised from 24 week old Wistar Kyoto rats (WKY) and Spontaneously Hypertensive rats (SHR) administered either no resveratrol (RSV) (No-RSV) or a high (100x) RSV dose (High-RSV) in the drinking water for 28 days. Data represent means ± S.E.M. See Table 2 and RESULTS for details.

**Fig. 4.** Peak magnitude of contraction (expressed relative to a previous KCl reference contraction) stimulated by a maximal dose of acetylcholine (Ach; -4.0 Log M) in non-precontracted common carotid artery rings (2 mm axial length; n = 4 per condition) excised from 20-24 week old Spontaneously Hypertensive rats and pre-incubated in-bath for 30 minutes with Nω-nitro-l-arginine methyl ester (l-NAME; 100 µM) plus either no resveratrol (RSV) (0 µM RSV), 1 µM RSV, 3 µM RSV, 10 µM RSV, 20 µM RSV, or 100 µM RSV. * p<0.05 denotes a significant difference between in-bath conditions. See RESULTS for details.
Fig. 5. Panel A depicts the dose-dependent vasocontractile response stimulated by cumulative Ach in non-precontracted common carotid arterial rings (n = 3-6 per condition) excised from 20-24 week old Spontaneously Hypertensive rats and pre-incubated in-bath for 30 minutes with \( N^\omega \)-nitro-\( l \)-arginine methyl ester (\( l \)-NAME; 100 \( \mu \)M) or \( l \)-NAME plus either RSV (20 \( \mu \)M, because this was the lowest concentration to elicit maximal attenuation of Ach-stimulated contraction, as depicted in Fig. 4) (\( l \)-NAME + RSV-20\( \mu \)M), the AMP-activated protein kinase (AMPK) activator 5-aminoimidazole-4-carboxamide (AICAR; 400 \( \mu \)M) (\( l \)-NAME + AICAR), the AMPK inhibitor Compound C (CC; 50 \( \mu \)M) (\( l \)-NAME + CC), AICAR + CC (\( l \)-NAME + CC + AICAR), or RSV and CC (\( l \)-NAME + RSV-20\( \mu \)M + CC). Panel B depicts the concentration of 6-keto prostaglandin (PG) F1\alpha (a stable product of PGI\(_2\)) in the bathing buffer surrounding \( l \)-NAME, \( l \)-NAME + RSV-20\( \mu \)M, \( l \)-NAME + AICAR, \( l \)-NAME + CC, \( l \)-NAME + CC + AICAR, or \( l \)-NAME + RSV-20\( \mu \)M + CC arterial rings following exposure to cumulative Ach in Panel A. * \( p<0.05 \) in Panel A denotes a significant difference among curve-fit parameters between in-bath conditions. * \( p<0.05 \) in Panel B denotes a significant difference between in-bath conditions. See Table 3 and RESULTS for details.
Fig. 6. Vasoconstriction stimulated by cumulative U46619 (a thromboxane-prostanoid receptor agonist) in the same non-precontracted common carotid arterial rings (n = 3-6 per condition) excised from 20-24 week old Spontaneously Hypertensive rats as those represented in Fig. 5A and 5B (following exposure to cumulative Ach, rings were washed thoroughly to re-establish a quiescent baseline for pre-incubation). Rings were pre-incubated in-bath for 30 minutes with $N^\omega$-nitro-$L$-arginine methyl ester ($L$-NAME; 100 $\mu$M) or $L$-NAME plus either 20 $\mu$M RSV ($L$-NAME + RSV-20$\mu$M), the AMP-activated protein kinase (AMPK) activator 5-aminoimidazole-4-carboxamide (AICAR; 400 $\mu$M) ($L$-NAME + AICAR), the AMPK inhibitor Compound C (CC; 50 $\mu$M) ($L$-NAME + CC), AICAR + CC ($L$-NAME + CC + AICAR), or RSV and CC ($L$-NAME + RSV-20$\mu$M + CC). * p<0.05 denotes a significant difference among curve-fit parameters between in-bath conditions. See Table 3 and RESULTS for details.
**TABLE 1**: Physical characteristics of WKY and SHR in relation to chronic in vivo RSV treatment.

<table>
<thead>
<tr>
<th>EFFECT</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No-RSV</td>
<td>Mod-RSV</td>
</tr>
<tr>
<td></td>
<td>No-RSV</td>
<td>Mod-RSV</td>
</tr>
<tr>
<td>Final Body Weight, g</td>
<td>313±10</td>
<td>314±5</td>
</tr>
<tr>
<td></td>
<td>346±5</td>
<td>329±3</td>
</tr>
<tr>
<td>Body Weight Change, g</td>
<td>23±7</td>
<td>15±6</td>
</tr>
<tr>
<td></td>
<td>19±5</td>
<td>20±2</td>
</tr>
<tr>
<td>Food Consumption, g • day⁻¹</td>
<td>17.4±0.4</td>
<td>17.5±0.3</td>
</tr>
<tr>
<td></td>
<td>19.4±0.4</td>
<td>18.5±0.2</td>
</tr>
<tr>
<td>Water Consumption, ml • day⁻¹</td>
<td>37±1</td>
<td>35±1</td>
</tr>
<tr>
<td></td>
<td>48±2</td>
<td>48±2</td>
</tr>
<tr>
<td>Calculated RSV intake, mg • day⁻¹ • kg body weight⁻¹</td>
<td>0.000</td>
<td>0.071±0.002*</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>0.093±0.004*</td>
</tr>
<tr>
<td>Mean Arterial Pressure, mmHg</td>
<td>78±6</td>
<td>82±7</td>
</tr>
<tr>
<td></td>
<td>176±7</td>
<td>172±9</td>
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</table>

Means ± S.E.M.; n=9-12 per condition, accept for mean arterial pressure which was an n=5-9 per condition. A p<0.05 was considered statistically significant. *, vs. No-RSV, and †, vs. Mod-RSV, denote statistical significance from post-hoc analysis of RSV main effect. WKY, Wistar Kyoto rat; SHR, Spontaneously Hypertensive rat; No-RSV, No Resveratrol; Mod-RSV, Moderate Resveratrol; High-RSV, High Resveratrol.
# Table 2: Curve-fit parameters of vasomotor function of CCA from WKY and SHR in relation to chronic in vivo RSV treatment.

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No-RSV (No Drug)</td>
<td>Mod-RSV (No Drug)</td>
</tr>
<tr>
<td>Ach-stimulated</td>
<td>Max Amp</td>
<td>87.0±4.9</td>
</tr>
<tr>
<td>relaxation in</td>
<td>EC50</td>
<td>-6.36±0.18</td>
</tr>
<tr>
<td>precontracted CCA</td>
<td>AUC</td>
<td>210±21</td>
</tr>
<tr>
<td>(see Fig. 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ach-stimulated</td>
<td>Max Amp</td>
<td>22.5±5.9</td>
</tr>
<tr>
<td>contraction in</td>
<td>EC50</td>
<td>-4.96±0.22</td>
</tr>
<tr>
<td>non-precontracted CCA</td>
<td>AUC</td>
<td>29±9</td>
</tr>
<tr>
<td>(see Fig. 2A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U46619-stimulated</td>
<td>Max Amp</td>
<td>130.5±9.4</td>
</tr>
<tr>
<td>contraction in</td>
<td>EC50</td>
<td>-8.23±0.02</td>
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<tr>
<td>non-precontracted CCA</td>
<td>AUC</td>
<td>290±24</td>
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<tr>
<td>(see Fig. 3)</td>
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Means ± S.E.M.; n=3-8 per condition. A p<0.05 was considered statistically significant. *, vs. No-RSV or Mod-RSV, denote statistical significance from post-hoc analysis of main effect. WKY, Wistar Kyoto rat; SHR, Spontaneously Hypertensive rat; No-RSV, No Resveratrol; Mod-RSV, Moderate Resveratrol; High-RSV, High Resveratrol.
TABLE 3. Curve-fit parameters of vasomotor function of SHR CCA in relation to acute in vitro RSV, AICAR, and/or CC treatment.

<table>
<thead>
<tr>
<th>IN-BATH DRUG CONDITION</th>
<th>EFFECT</th>
<th>p value</th>
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</tr>
<tr>
<td>L-NAME</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max Amp</td>
<td>62.1±1.6</td>
<td>6.9±4.3*</td>
</tr>
<tr>
<td>EC50</td>
<td>-5.4±0.12</td>
<td>-5.7±0.34</td>
</tr>
<tr>
<td>AUC</td>
<td>92±8</td>
<td>11±6*</td>
</tr>
<tr>
<td>L-NAME + RSV-20µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max Amp</td>
<td>170.5±3.6</td>
<td>134.0±4.9*</td>
</tr>
<tr>
<td>EC50</td>
<td>-7.91±0.09</td>
<td>-8.32±0.11</td>
</tr>
<tr>
<td>AUC</td>
<td>320±14</td>
<td>215±14*</td>
</tr>
<tr>
<td>L-NAME + AICAR</td>
<td></td>
<td></td>
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<tr>
<td>Max Amp</td>
<td>137.1±9.1</td>
<td>154.8±12.5</td>
</tr>
<tr>
<td>EC50</td>
<td>-8.32±0.11</td>
<td>-8.03±0.15</td>
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<tr>
<td>AUC</td>
<td>308±16</td>
<td>309±34</td>
</tr>
<tr>
<td>L-NAME + CC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max Amp</td>
<td>154.8±12.5</td>
<td>150.4±18.0</td>
</tr>
<tr>
<td>EC50</td>
<td>-8.03±0.15</td>
<td>-8.28±0.13</td>
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<tr>
<td>AUC</td>
<td>309±34</td>
<td>336±37</td>
</tr>
<tr>
<td>L-NAME + AICAR + CC</td>
<td></td>
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<tr>
<td>Max Amp</td>
<td>150.4±18.0</td>
<td>150.4±18.0</td>
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<tr>
<td>EC50</td>
<td>-8.28±0.13</td>
<td>-8.28±0.13</td>
</tr>
<tr>
<td>AUC</td>
<td>336±37</td>
<td>336±37</td>
</tr>
<tr>
<td>L-NAME + RSV-20µM + CC</td>
<td></td>
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</tr>
<tr>
<td>Max Amp</td>
<td>122.7±7.1*</td>
<td>122.7±7.1*</td>
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<tr>
<td>EC50</td>
<td>-7.69±0.12</td>
<td>-7.69±0.12</td>
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<tr>
<td>AUC</td>
<td>205±15*</td>
<td>205±15*</td>
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Means ± S.E.M.; n=3-8 per condition. A p<0.05 was considered statistically significant. *, vs. L-NAME, denotes statistical significance from post-hoc analysis of main effect. L-NAME, Nω-nitro-L-arginine methyl ester; RSV, Resveratrol; AICAR, 5-Aminoimidazole-4-Carboxamide; CC, Compound C.
% relaxation (from 10^-6 M phenylephrine precontraction) vs Ach (Log M)

WKY

No-RSV (No Drug)
Mod-RSV (No Drug)
High-RSV (No Drug)
No-RSV (Indo)

SHR

No-RSV (No Drug)
Mod-RSV (No Drug)
High-RSV (No Drug)
No-RSV (Indo)
Ach (Log M) % of 60 mM KCl contraction

A

B

6-keto PGF1α (pg x ml⁻¹)

L-NAME
L-NAME + RSV-20 μM
L-NAME + AICAR
L-NAME + CC
L-NAME + AICAR + CC
L-NAME + RSV-20 μM + CC

* *
% of 60 mM KCl contraction

-4.0
-3.0
-2.0
-1.0
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0

U46619 (Log M)

L-NAME
L-NAME + RSV-20uM
L-NAME + AICAR
L-NAME + CC
L-NAME + AICAR + CC
L-NAME + RSV-20uM + CC

SHR

* *