

Effect of Atenolol or Nebivolol Administration on Blood Pressure Variability and its Impact on White Organ Damage in Spontaneously Hypertensive Rats

Efecto de la administración de atenolol o nebivolol sobre la variabilidad de la presión arterial y su impacto sobre el daño de órgano blanco en ratas espontáneamente hipertensas

JULIETA S. DEL MAURO¹, PAULA D. PRINCE², NAHUEL FERNÁNDEZ MACHULSKY³, MARCELA A. MORETTON⁴, MARTÍN DONATO⁵, GERMÁN E. GONZÁLEZ⁵, CELINA MORALES^{MTSAC, 5}, RICARDO J. GELPI^{MTSAC, 5}, CARLOS A. TAIRA^{1, 6}, CHRISTIAN HÖCHT^{1, 6}

ABSTRACT

Background: Hypertension-related cardiovascular risk has been traditionally considered the result of sustained blood pressure elevation, leading to target organ injury. In recent years, other factors have been described, such as blood pressure and central blood pressure variability, also directly affecting target organ injury.

Objective: The aim of this study was to assess the effects of chronic atenolol or nebivolol administration on short-term blood pressure variability and target organ injury at the left ventricular and aortic level.

Method: Spontaneously hypertensive rats (SHR) were treated for 8 weeks with a single daily dose of atenolol, nebivolol or vehicle. Blood pressure and its short-term variability were measured and echocardiography was performed. The left ventricle and thoracic aorta were removed to quantify the expression of transforming growth factor β and to perform histological studies.

Results: Chronic treatment with nebivolol reduced mean arterial pressure (MAP) and its variability to a greater extent than atenolol (MAP: WKY: 118.6 ± 8.0 mmHg, SHR: 174.6 ± 2.1 mmHg a; SHR-atenolol: 155.2 ± 2.1 mmHg a, b, SHR-nebivolol: 122.3 ± 2.3 mmHg b, c, MAP variability: WKY: 3.8 ± 0.2 mmHg; SHR: 6.2 ± 0.3 mmHg a; SHR-atenolol: 5.2 ± 0.3 mmHg a, b; SHR-nebivolol: 4.2 ± 0.2 mmHg b, c; a $p < 0.05$ vs. WKY rats; b $p < 0.05$ vs. SHR; c $p < 0.05$ vs. SHR-atenolol).

Conclusions: The analysis of target organ injury showed that nebivolol reduced the content of ventricular fibrosis, decreased aortic media thickness and induced greater reduction of transforming growth factor β overexpression in both organs compared to SHR treated with vehicle or atenolol. These findings suggest that a greater reduction in central blood pressure, together with a decrease in blood pressure variability, would contribute to the better protection of target organ injury with nebivolol than with atenolol.

Key words: Nebivolol - Atenolol - Blood Pressure - Rats

RESUMEN

Introducción: Tradicionalmente se ha considerado que el riesgo cardiovascular asociado con la hipertensión es producto de la elevación sostenida de la presión arterial, que lleva al daño de órgano blanco. En los últimos años se ha descrito que otros factores, como la variabilidad de la presión arterial y la presión arterial central, también afectan directamente el daño de órgano blanco.

Objetivo: Determinar los efectos de la administración crónica de atenolol o nebivolol sobre la variabilidad de la presión arterial a corto plazo y el daño de órgano blanco a nivel del ventrículo izquierdo y de la aorta.

Material y métodos: Se incluyeron ratas espontáneamente hipertensas (REH) que fueron tratadas durante 8 semanas con una única administración diaria de atenolol, nebivolol o vehículo. Se determinaron la presión arterial y su variabilidad a corto plazo y se realizó ecocardiografía. Se extrajeron el ventrículo izquierdo y la aorta torácica para cuantificar la expresión del factor de crecimiento transformante β y realizar estudios histológicos.

REV ARGENT CARDIOL 2017;85:84-89. <http://dx.doi.org/10.7775/rac.v85.i2.10197>

Received: 01/25/2017 – Accepted: 03/02/2017

Address for reprints: Julieta Sofia del Mauro - Cátedra de Farmacología. Facultad de Farmacia y Bioquímica- Universidad de Buenos Aires - Junín 956 - (C1113AAD) Buenos Aires, Argentina - Tel. +(54-11)-4964-8265 - Fax +(54-11)-4508-3645 - e-mail: julietadelmauro@gmail.com

MTSAC Full Member of the Argentine Society of Cardiology

¹ Department of Pharmacology, School of Pharmacy and Biochemistry, Universidad de Buenos Aires. Buenos Aires, Argentina

² Chair of Physical Chemistry, Institute of Biochemistry and Molecular Medicine (IBIMOL), School of Pharmacy and Biochemistry, Universidad de Buenos Aires. Buenos Aires, Argentina

³ Laboratory of Lipids and Atherosclerosis, Department of Clinical Biochemistry, INFIBIOC, School of Pharmacy and Biochemistry, Universidad de Buenos Aires, Buenos Aires, Argentina.

⁴ Department of Pharmaceutical Technology, School of Pharmacy and Biochemistry, Universidad de Buenos Aires - CONICET. Buenos Aires, Argentina.

⁵ Institute of Cardiovascular Physiopathology (INFICA), Department of Pathology, School of Medicine, Universidad de Buenos Aires - CONICET . Buenos Aires, Argentina.

⁶ Institute of Pathophysiology and Clinical Biochemistry, School of Pharmacy and Biochemistry, Universidad de Buenos Aires. Buenos Aires, Argentina

Resultados: El tratamiento crónico con nebivolol redujo la presión arterial media (PAM) y su variabilidad en mayor medida que el atenolol (PAM WKY: $118,6 \pm 8,0$ mm Hg; REH: $174,6 \pm 2,1$ mm Hg a; REH-atenolol: $155,2 \pm 2,1$ mm Hg a, b; REH-nebivolol: $122,3 \pm 2,3$ mm Hg b, c; desviación estándar de la PAM WKY: $3,8 \pm 0,2$ mm Hg; REH: $6,2 \pm 0,3$ mm Hg a; REH-atenolol: $5,2 \pm 0,3$ mm Hg a, b; REH-nebivolol: $4,2 \pm 0,2$ mm Hg b, c; a $p < 0,05$ vs. ratas WKY; b $p < 0,05$ vs. REH; c $p < 0,05$ vs. REH-atenolol).

Conclusiones: El análisis del daño de órgano blanco establece que el nebivolol reduce el contenido de fibrosis ventricular, disminuye el espesor de la media aórtica e induce una mayor reducción de la sobreexpresión del factor de crecimiento transformante β en ambos órganos en comparación con REH tratadas con vehículo o atenolol. Estos hallazgos sugieren que la mayor reducción de la presión arterial central, junto con la disminución de la labilidad de la presión arterial, contribuiría en la superior protección del daño de órgano blanco por el nebivolol respecto del atenolol.

Palabras clave: Nebivolol - Atenolol - Presión arterial - Ratas

Abbreviations

BP	Blood Pressure	SD	Standard Deviation
BPV	Blood Pressure Variability	SHR	Spontaneously hypertensive rats
ICF	Interstitial collagen fraction	TA	Thoracic artery
LV	Left Ventricle	TGF-β	Transforming growth factor β
MAP	Mean Arterial Pressure	WKY	Wistar Kyoto
SBP	Systolic Blood Pressure		

INTRODUCTION

Cardiovascular risk associated to hypertension has been traditionally considered as the product of sustained blood pressure (BP) elevation leading to target organ damage. In recent years other factors, such as blood pressure variability (BPV) and central blood pressure (BP), have also been described to directly affect target organ injury. (1-6) Different clinical studies have established that a 24 hour-increase in BPV, day-to-day and between medical visits is associated with an increased risk of cardiovascular events. (7) Today, it is postulated that the reduction of BPV may be considered as a possible new therapeutic goal for the treatment of hypertension, which is why antihypertensive drugs should not only be able to decrease BP but also to control its variability. (7)

Central BP represents the direct pressure exerted on the brain, heart and kidneys and has shown to have closer correlation with target organ damage and the incidence of cardiovascular events compared with brachial artery BP. (3, 4) A retrospective analysis showed that atenolol therapy, while reducing brachial artery BP, is capable of elevating central BP, generating a false sense of hypertension and cardiovascular risk control. (8, 9)

β -blockers are no longer recommended for initial treatment in uncomplicated hypertensive subjects due to their lower protection against stroke and the increased risk of metabolic disorders. (8) The lower cardioprotection provided by β -blockers compared to other first-line antihypertensive agents has been attributed to its limited effect on central BP and BPV (8, 10) However, the clinical evidence associating the use of β -blockers with lower protection of the hypertensive patient is largely based on clinical studies evaluating atenolol, a relatively low cardioselective β -blocker with no vasodilator action. (7, 8)

In the last decades, third-generation β -blockers, among them carvedilol and nebivolol, have been developed with pharmacological properties superior to atenolol, including the ability to reduce central BP and a neutral metabolic profile. (8, 11) However, the superiority of third generation β -blockers over atenolol in terms of BPV reduction and protection of target organ damage has not been previously evaluated in clinical studies.

Considering this background, the aim of the present study was to study the effects of chronic oral administration of atenolol or nebivolol on short-term BPV and to evaluate its action on target organ injury markers at the left ventricular (LV) and thoracic aorta (TA) level.

METHODS

Spontaneously hypertensive male rats (SHR) (200-220 g), were orally treated during 8 weeks with nebivolol 30 mg/kg/day (n=6), atenolol 90 mg/kg/day (n=6) or vehicle, using a trocar. A fourth group of WKY rats treated with vehicle was employed as control. During the last 2 weeks of treatment, systolic blood pressure (SBP) was assessed by the indirect tail-cuff method, estimating mean SBP, intraday variation and inter-day fluctuation. (12)

In the last week of treatment, echocardiographic measurements were performed with an Acuson Sequoia, model C512 ultrasound system equipped with a 7-14 MHz transducer in rats anesthetized with ketamine/xylazine. After 2 months of treatment, the carotid artery was cannulated and connected 24 hours later to a Spectramed P23XL pressure transducer (Spectramed, Oxnard, CA, USA) coupled to a Grass 79D polygraph (Grass Instruments, Quincy, MA, USA) and a digital converter (Polyview, PVA 1, Grass-Astro Med, West Warwick, RI USA). Blood pressure recordings were obtained during 2 hours and analyzed using Polyview 2.3 software (Astro-Med, West Warwick, RI, USA). Mean arterial pressure (MAP), which for its sampling site represents central BP, was estimated and short term BPV was calculated from 3-minute recordings. (13)

Once the hemodynamic parameters were measured, the animals were sacrificed by guillotine decapitation removing the TA and the LV to assess target organ damage. As a morphological marker of injury at the cardiac level, the left ventricular weight/body weight ratio was evaluated using a precision weigh scale. (14) The histopathological evaluation of the LV and the TA was performed on 5 μ m thick tissue sections. The aortas were stained with hematoxylin-eosin (H&E) while the LVs with Sirius Red stain in which the collagen fibers are stained pink. Microscopic sections were photographed using a microscope (Olympus CX31 microscope, Japan) attached to a digital camera (U-CMA D3 Olympus, Japan). At the ventricular level, the interstitial collagen fraction (ICF) was determined, while at the aortic level the aortic media thickness and the aortic media thickness/lumen diameter ratio were evaluated using image analyzer software (Image Pro-Plus 3.0, Media Cybernetics, Silver Spring, MD, USA for the LV and ImageJ 1.49 V Wayne Rasband, National Institute of Health, USA for the aortas). (15, 16) Finally, the transforming growth factor- β (TGF- β) expression in the LV and TA homogenates was assessed by Western Blot analysis as biochemical marker of target organ damage. Homogenates (20 μ g) were run on SDS polyacrylamide gel (12%). The transfer was performed on PVDF membranes which, after blockage, were incubated overnight with: Mouse anti-TGF- β (MW:13 kDa) and rabbit anti-GAPDH (MW:37 kDa) as loading control. Proteins were detected by chemiluminescence using a photographic film and then quantified with the ImageJ program. (17)

Statistical analysis

Normal distribution of study data and variables was confirmed using the Kolmogorov Smirnov test. Data were expressed as mean \pm standard error of the mean. Statistical comparison between treatment groups was performed using one-way ANOVA followed by Tukey's post-hoc test using GraphPad Prism version 6.0 (GraphPad Software, San Diego, California, USA). Statistical significance was defined for a p value <0.05.

Ethical considerations

Animal experiments were performed according to the "Principles of Care and Use of Laboratory Animals" (NIH

Publication No. 85-3, Revision 1985) and were approved by the Ethics Committee of the School of Pharmacy and Biochemistry of Universidad de Buenos Aires (EXP-UBA N° 0062949/2015).

RESULTS

Chronic treatment with nebivolol, while not significantly reducing SBP, decreased intraday and between days fluctuation compared with hypertensive animals treated with vehicle or atenolol (Table 1). Central BP assessment showed that nebivolol reduced MAP and its short-term variability to a greater extent than atenolol, restoring the values of the normotensive control group (Table 1).

Echocardiographic findings revealed a significant increase of the posterior wall diameter during diastole in SHR compared with normotensive animals, which was reduced to a greater extent by nebivolol compared with atenolol (-29% vs. -8%, $p < 0.05$). Chronic nebivolol administration reduced left ventricular mass index expressed as LV weight/body weight ratio (Table 1). The histopathological evaluation established the existence of an increase in LV interstitial collagen deposition of SHR compared with normotensive animals, evidenced by 62% increase in the ICF. Although atenolol partially reduced the interstitial collagen content, nebivolol completely reversed this cardiac structure alteration, reducing the LV fibrosis content in SHR. The analysis of TA histological sections showed an increase in both the aortic media thickness and aortic media thickness/lumen diameter in SHR compared with WKY rats. Both morphometry parameters were reduced only after chronic treatment with nebivolol (Table 2). Finally, the biochemical evaluation of target organ damage showed that nebivolol induces a greater reduction of TGF- β overexpression compared with atenolol, both at the ventricular and aortic levels (Figure 1A).

Table 1. Hemodynamic parameters of WKY rats and SHR chronically treated with atenolol, nebivolol or vehicle.

Hemodynamic parameter	WKY (n=6)	SHR (n=6)	SHR Atenolol (n=6)	SHR Nebivolol (n=6)
Indirect pressure (tail cuff)				
SBP, mmHg	134 \pm 2	184 \pm 6 ^a	169 \pm 2 ^{a,b}	170 \pm 4 ^a
Intraday SD, mmHg	3.38 \pm 0.52	9.21 \pm 0.59 ^a	6.42 \pm 0.35 ^{a,b}	3.76 \pm 0.40 ^{b,c}
Interday SD, mmHg	3.04 \pm 0.75	10.42 \pm 0.80 ^a	13.90 \pm 2.08 ^a	7.30 \pm 0.84 ^{b,c}
Central blood pressure in the carotid artery				
MAP, mmHg	118.6 \pm 8.0	174.6 \pm 2.1 ^a	155.2 \pm 2.1 ^{a,b}	122.3 \pm 2.3 ^{b,c}
SD, mmHg	3.8 \pm 0.2	6.2 \pm 0.3 ^a	5.2 \pm 0.3 ^{a,b}	4.2 \pm 0.2 ^{b,c}
Echocardiography				
Diastolic posterior wall diameter, mm	1.31 \pm 0.08	2.40 \pm 0.03 ^a	2.20 \pm 0.01 ^a	1.70 \pm 0.04 ^{a,b}
Left ventricular mass index mg/g	2.17 \pm 0.10	3.51 \pm 0.18 ^a	3.52 \pm 0.02 ^a	2.98 \pm 0.06 ^{a,b,c}

The data are expressed as mean \pm standard error of the mean. ^a $p < 0.05$ vs. WKY, ^b $p < 0.05$ vs. SHR, ^c $p < 0.05$ vs. SHR Atenolol. WKY: Wistar Kyoto. SHR: Spontaneously hypertensive rats. SBP: Systolic blood pressure. SD: Standard deviation. MAP: Mean arterial pressure

DISCUSSION

In the present study, chronic oral treatment with the third-generation β -blocker vasodilator nebivolol markedly attenuates short-term BPV in the SHR model. The analysis of direct and indirect BP measurements establishes that nebivolol at a daily dose of 30 mg/kg is able to significantly reduce the SD of the BP registry, a recognized parameter of short-term variability. At an indirect level, although nebivolol does not generate a reduction in SBP, it reduces the short and mid-term fluctuation parameters of BP. The different efficacy of some drugs in terms of decreased central BP compared with peripheral BP may be one of the causes why agents that produce similar reductions in peripheral blood pressure do not achieve equal protection against cardiovascular adverse events. (13) Nebivolol is more effective in reducing central BP and its variability than atenolol, suggesting that β -blocker vasodilators provide a greater hemodynamic benefit compared to second-generation β -blockers.

Currently, the effect of nebivolol on BPV has been scarcely reported in preclinical studies. In previous works, we have demonstrated that the acute intravenous administration of nebivolol in SHR, in L-NAME induced hypertensive rats, and in normotensive rats but with increased fluctuation of BP by means of sinus aortic denervation, substantially reduces short-term BPV. (13, 18, 19) At the clinical level, Gapon et al. have shown that, although both atenolol and nebivolol exhibit similar antihypertensive properties, treatment with nebivolol prevents the increase in morning BP and its variability to a greater extent than atenolol over 24 hours in patients with second degree hypertension. (20)

A second objective of the present study was to compare the effect of chronic treatment with nebivolol or atenolol on target organ damage at the LV and TA levels in SHR, by morphological, echocardiographic, histological, and biochemical determinations. Only the third-generation β -blocker was able to decrease TGF- β overexpression, a cytokine involved in fibrotic processes implicated in cardiac and vascular remodeling during hypertension. (21, 22) Ac-

cordingly, chronic antihypertensive treatment with nebivolol was able to protect against the ventricular and aortic remodeling characteristic of hypertensive disease. In this context, nebivolol reduced the interstitial collagen content, the ventricular hypertrophy index and the posterior diastolic wall thickness found in hypertensive animals treated with vehicle or atenolol. Recently, Varagic et al. revealed that nebivolol attenuates remodeling and cardiac dysfunction in salt-sensitive SHR through an independent effect of its antihypertensive action. (23) At the vascular level, chronic treatment with nebivolol was able to reverse aortic remodeling evidenced by a reduction in the media layer thickness and the media thickness/lumen diameter ratio. These results are in agreement with the findings of Guerrero et al. showing that chronic treatment with nebivolol reduces the fibrotic content and improves vascular structure. (24)

CONCLUSIONS

Essentially, chronic administration of nebivolol presents greater hemodynamic effects than atenolol, evidenced by a higher reduction of central BP and short-term BPV. Nebivolol is capable of attenuating ventricular and vascular injury associated with experimental hypertension to a greater extent than atenolol. These results suggest that the lower cardiovascular protection of atenolol compared with other antihypertensive agents found in clinical trials should not be extrapolated to third-generation β -blockers such as nebivolol.

Acknowledgments

This work was supported by scholarships from the Secretariat of Science and Technology, Universidad de Buenos Aires, Argentina. Martín Donato, Germán E. González and Carlos A. Taira are CONICET career researchers.

Conflicts of interest

None declared.

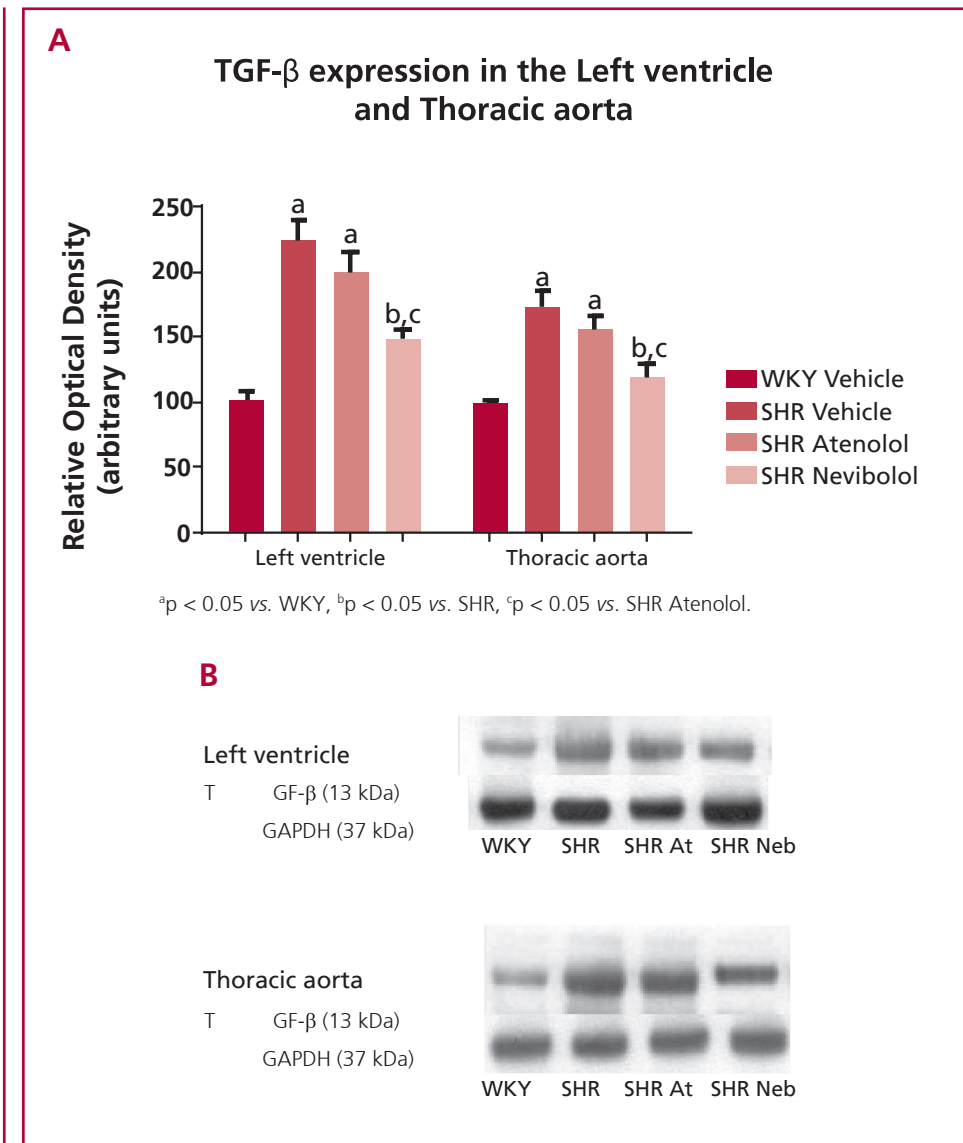
(See authors' conflicts of interest forms on the website/Supplementary material).

	WKY (n=6)	SHR (n=6)	SHR Atenolol (n=6)	SHR Nebivolol (n=6)
Left ventricle (Sirius Red)				
ICF, %	3.41±0.11	5.53±0.17 ^a	4.50±0.13 ^{a,b}	3.14±0.34 ^{b,c}
Thoracic aorta (H&E)				
Aortic media thickness, μ m	103.0±4.9	151.2±3.0 ^a	145.4±2.1 ^a	106.2±2.1 ^{b,c}
Aortic media thickness /lumen diameter, μ m/mm	58.6±3.5	91.6±2.7 ^a	94.3±2.2 ^a	74.2±3.6 ^{a,b,c}

The data are expressed as mean±standard error of the mean. ^ap<0.05 vs. WKY, ^bp<0.05 vs. SHR, ^cp<0.05 vs. SHR Atenolol. WKY: Wistar Kyoto. SHR: Spontaneously hypertensive rats. SBP: Systolic blood pressure. ICF: Interstitial collagen fraction. H&E: Hematoxylin-eosin.

Table 2. Histological left ventricular and thoracic aorta results of WKY and SHR rats chronically treated with atenolol, nebivolol or vehicle.

Fig. 1. Beta transforming factor (TGF- β) expression (A) and its representative images (B) of Western blot analysis in the left ventricle and thoracic aorta of Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHR) chronically treated with atenolol, nebivolol or vehicle. Data are expressed as optical density relative to WKY control animals. At: Atenolol. Neb: Nebivolol. (GAPDH): glyceraldehyde 3-phosphate dehydrogenase.



REFERENCES

- Parati G, Liu X, Ochoa JE. Clinical relevance of visit-to-visit blood pressure variability: impact on renal outcomes. *J Hum Hypertens* 2014;28:403-9. <http://doi.org/f579cm>
- Höcht C, Del Mauro JS, Bertera FM, Taira CA. Drugs affecting blood pressure variability: an update. *Curr Pharm Des* 2015;21:744-55. <http://doi.org/97v>
- Roman MJ, Devereux RB. Association of central and peripheral blood pressures with intermediate cardiovascular phenotypes. *Hypertension* 2014;63:1148-53. <http://doi.org/b63j>
- Trudeau L. Central blood pressure as an index of antihypertensive control: determinants and potential value. *Can J Cardiol* 2014;30(5 Suppl):S23-8. <http://doi.org/f5x667>
- Grassi G, Bombelli M, Brambilla G, Trevano FQ, Dell'oro R, Mancia G. Total cardiovascular risk, blood pressure variability and adrenergic overdrive in hypertension: evidence, mechanisms and clinical implications. *Curr Hypertens Rep* 2012;14:333-8. <http://doi.org/2q9>
- Parati G. Blood pressure variability: its measurement and significance in hypertension. *J Hypertens Suppl* 2005;23:S19-25. <http://doi.org/frsbsj>
- Höcht C. Blood pressure variability: prognostic value and therapeutic implications. *ISRN Hypertension* 2013;ID 398485.
- Ripley TL, Saseen JJ. β -blockers: a review of their pharmacological and physiological diversity in hypertension. *Ann Pharmacother* 2014;48:723-33. <http://doi.org/2rb>
- DiNicolantonio JJ, Hackam DG. Carvedilol: a third-generation β -blocker should be a first-choice β -blocker. *Expert Rev Cardiovasc Ther* 2012;10:13-25. <http://doi.org/c45trg>
- Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, et al; ASCOT-BPLA and MRC Trial Investigators. Effects of beta blockers and calcium-channel blockers on within-individual variability in blood pressure and risk of stroke. *Lancet Neurol* 2010;9:469-80. <http://doi.org/fk7pth>
- Dhakam Z, Yasmin, McEniery CM, Burton T, Brown MJ, Wilkinson IB. A comparison of atenolol and nebivolol in isolated systolic hypertension. *J Hypertens* 2008;26:351-6. <http://doi.org/c9jn53>
- Kurtz TW, Griffin KA, Bidani AK, Davisson RL, Hall JE; Subcommittee of Professional and Public Education of the American Heart Association. Recommendations for blood pressure measurement in humans and experimental animals. Part 2: Blood pressure measurement in experimental animals: a statement for professionals from the subcommittee of professional and public education of the American Heart Association council on high blood pressure re-

search. *Hypertension* 2005;45:299-310. <http://doi.org/d6m94h>

13. Bertera FM, Del Mauro JS, Lovera V, Chiappetta D, Polizio AH, Taira CA, et al. Acute effects of third generation β -blockers on short-term and beat-to-beat blood pressure variability in sinoaortic-denervated rats. *Hypertens Res* 2013;36:349-55. <http://doi.org/2rc>
14. Miao CY, Xie HH, Zhan LS, Su DF. Blood pressure variability is more important than blood pressure level in determination of end-organ damage in rats. *J Hypertens* 2006;24:1125-35. <http://doi.org/cjsf77>
15. González GE, Cassaglia P, Noli Truant S, Fernández MM, Wilensky L, Volberg V, et al. Galectin-3 is essential for early wound healing and ventricular remodeling after myocardial infarction in mice. *Int J Cardiol* 2014;176:1423-5. <http://doi.org/b63k>
16. Xiong X, You C, Feng QC, Yin T, Chen ZB, Ball P, et al. Pulse with modulation electro-acupuncture on cardiovascular remodeling and plasma nitric oxide in spontaneously hypertensive rats. *Evid Based Complement Alternat Med* 2011;2011:812160. doi:10.1093/ecam/nej06
17. Ceron CS, Rizzi E, Guimarães DA, Martins-Oliveira A, Gerlach RF, Tanus-Santos JE. Nebivolol attenuates prooxidant and profibrotic mechanisms involving TGF- β and MMPs, and decreases vascular remodeling in renovascular hypertension. *Free Radic Biol Med* 2013;65:47-56.
18. Bertera FM, Del Mauro JS, Lovera V, Chiappetta D, Polizio AH, Taira CA, et al. Enantioselective pharmacokinetics and cardiovascular effects of nebivolol in L-NAME hypertensive rats. *Hypertens Res* 2014;37:194-201. <http://doi.org/wp5>
19. Bertera FM, Del Mauro JS, Polizio AH, Chiappetta D, Taira CA, Höcht C. Effect of nebivolol on beat-to-beat and short-term blood pressure variability in spontaneously hypertensive rats. *Naunyn Schmiedebergs Arch Pharmacol* 2012;385:833-43. <http://doi.org/wp6>
20. Gapon L, Prilepova AA, Tsygol'nik MD. Effect of nebivolol on parameters of cerebral haemodynamics and 24-hour blood pressure profile in patients with arterial hypertension. *Kardiologiya* 2005;45:18-22.
21. Sun L, Jin H, Sun L, Chen S, Huang Y, Liu J, et al. Hydrogen sulfide alleviates myocardial collagen remodeling in association with inhibition of TGF- β /Smad signaling pathway in spontaneously hypertensive rats. *Mol Med* 2015;20:503-15.
22. Shang P, Liu W, Liu T, Zhang Y, Mu F, Zhu Z, et al. Acetyl-11-keto- β -boswellic acid attenuates prooxidant and profibrotic mechanisms involving transforming growth factor- β 1, and improves vascular remodeling in spontaneously hypertensive rats. *Sci Rep* 2016;6:39809. <http://doi.org/f9j35v>
23. Varagic J, Ahmad S, Voncannon JL, Moniwa N, Simington SW Jr, Brosnihan BK, et al. Nebivolol reduces cardiac angiotensin II, associated oxidative stress and fibrosis but not arterial pressure in salt-loaded spontaneously hypertensive rats. *J Hypertens* 2012;30:1766-74.
24. Guerrero EI, Ardanaz N, Sevilla MA, Arévalo MA, Montero MJ. Cardiovascular effects of nebivolol in spontaneously hypertensive rats persist after treatment withdrawal. *J Hypertens* 2006;24:151-8. <http://doi.org/djtxg>