British Journal of Medicine & Medical Research
18(9): 1-8, 2016, Article no.BJMMR.28626
ISSN: 2231-0614, NLM ID: 101570965

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effects of Racemic Nebivolol and Its Stereoisomers on Rat Aortic Segments in Two Age Groups

Ulya Keskin[1], Oguzhan Ekin Efe[1*] and Meral Tuncer[1]

[1]Department of Pharmacology, Faculty of Medicine, Baskent University, Turkey.

Authors' contributions

This work was carried out in collaboration between all authors. All authors played role in designing the study, writing the protocol and writing the first draft of the manuscript. And they also together managed the literature searches, analyses of the study, performed the spectroscopy analysis, managed the experimental process and identified the species of plant. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/28626
Editor(s):
(1) Shatrah Binti Othman, Department of Molecular Medicine, University of Malaya, Kuala Lumpur, Malaysia.
(2) Chan-Min Liu, School of Life Science, Xuzhou Normal University, Xuzhou City, China.
Reviewers:
(1) Michael Chen, California State University, USA.
(2) Pius M. Udia, University of Calabar, Nigeria.
(3) Bopda Mtopi Orelien Sylvain, University of Buea, Cameroon.
(4) Aino Siltari, University of Helsinki, Finland.
Complete Peer review History: http://www.sciencedomain.org/review-history/16867

Original Research Article

Received 28th July 2016
Accepted 10th October 2016
Published 10th November 2016

ABSTRACT

Objectives: We aimed to investigate the effects of racemic, D- and L-nebivolol in aortic segments obtained from rats of two ages to determine whether age- or stereoisomer-related differences occurred in the relaxation of aortic segments contracted with phenylephrine.
Background: Nebivolol, an antihypertensive drug with reported beta adrenergic receptor blocking effects, is clinically administered as a racemic mixture with D and L isomers. The D isomer of nebivolol is more selective than the L isomer for blocking beta-1 adrenergic receptors, whereas L-nebivolol produces greater nitric oxide (NO)-mediated vasodilation.
Methods: Aortic segments were isolated from male Wistar rats aged 4 and 8 months. Relaxations were obtained with three forms of nebivolol. The protocol was repeated following preincubation with NG-nitro-L-arginine methyl ester (L-NAME) alone or with indomethacin.
Results: A significant age-related difference in relaxation was found only for racemic nebivolol and only at a concentration of 10^{-4} M. L-NAME and indomethacin did not affect the relaxations. Nebivolol noncompetitively shifted the phenylephrine concentration-response curve.

**Corresponding author: E-mail: drekinefe@gmail.com;*

Conclusion: Relaxation by nebivolol was not dependent on age or stereoisomer. Neither cyclooxygenase metabolites nor NO played a role in relaxations. Results suggest that inhibition of phenylephrine contractions by nebivolol is due to blockade of alpha adrenergic receptor activity.

Keywords: Nebivolol and stereoisomers; nitric oxide (NO); L-NAME; indomethacin; phenylephrine.

1. INTRODUCTION

Drug effects may differ in young and elderly people because of age-related changes in drug pharmacokinetics, decreases in drug receptor number and sensitivity, and increases in the number of coincident or previous diseases, as well as endothelial dysfunction with age [1]. Conflicting results have been reported in the levels and effects of some agonists in aging. For example, although a decrease in arterial noradrenaline content was detected in some studies [2-4], the vasoconstrictor responses to noradrenaline did not differ with age [5-9]. By contrast, it was observed that there was an age-related decrease in both the arterial noradrenaline content and sensitivity to exogenous noradrenaline [9]. In contrast to these conflicting results for the noradrenergic system, several studies have shown that nitric oxide (NO)-mediated vasodilation is impaired and total body NO production is decreased in elderly people [10,11]. For example, NO-mediated vasodilation in the forearm vascular bed of humans was shown to decrease in an older population, reflecting a decrease in NO synthesis [12,13].

Nebivolol is a relatively new antihypertensive drug with β1 adrenoceptor blocking activity. Nebivolol decreases peripheral vascular resistance by producing vasodilation in patients with hypertension. In addition to its β1 receptor blocking activity, nebivolol also affects endothelial nitric oxide release [14], β2 adrenergic receptor blockade [15] and calcium-activated potassium channels activation [16], all of which may play a role in the vasodilator effect of this drug. In addition to these actions, In addition to these actions, it has been reported that nebivolol-induced relaxation results from both inhibition of α1-adrenergic receptors and activation of β3-adrenergic receptors in rat aorta [17]. However, it has also been reported that potassium channels do not mediate the vasodilative effect of nebivolol on different types of rat arteries [18]. Nebivolol is a racemic mixture, with equal proportions of both stereoisomers. The D-isomer has more selective β1-blocker activity than the L-isomer [19], whereas L-nebivolol produces NO-mediated vasodilation and stimulates the endothelial NO synthase (eNOS) enzyme to a greater degree than D-nebivolol [19]. The vasodilator effect of nebivolol is antagonized by the NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) [20].

The aim of the present study was to investigate the effects of racemic, D-, and L-nebivolol in aortic segments obtained from rats of different ages to determine whether an age- or stereoisomer-related difference occurred in the contraction induced by phenylephrine, a selective α1-adrenergic receptor agonist, or NO-mediated relaxation. Our hypothesis was that the L-isomer would produce greater vasodilation than the D-isomer in vessels precontracted by phenylephrine in the younger animals. Additionally, we examined the effect of nebivolol on the phenylephrine concentration-response curve because we found no contraction of the aortic segments with phenylephrine treatment following the administration of high doses of nebivolol.

2. MATERIALS AND METHODS

This study was approved by the Baskent University Ethical Committee for Experimental Research on Animals (Project no: DA 12/07).

2.1 Animals

In this study, male Wistar albino rats of two ages were used: Group I was 4 months old (body weight 200–230 g; n = 8) and Group II was 8 months old (body weight 350–400 g; n = 8).

2.2 Tissue Preparation and Tension Measurement in Rat Aortic Rings

Cervical dislocation was applied under anesthesia (ketamine, 60 mg/kg and xylazine, 10 mg/kg). Following an abdominal incision along the middle axis, the thoracic space was opened. The thoracic aorta was removed, isolated from the surrounding tissue, and cut into six segments of precisely 3 mm in length. For standardization, segments to a distance of 10 mm from out of the heart were studied. Thus, a total of 96 segments (6 segments from 16 animals) were studied. Isolated aortic rings were mounted in organ chambers filled with Krebs-Henseleit solution

(118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 10 mM glucose) at 37℃ and continuously gassed with a mixture of 95% O_2 and 5% CO_2. The preparations were attached to force-displacement transducers (MP100, BioPac Systems, Inc., COMMAT Ltd., Turkey) that were connected to a computer for isometric tension recording and processing. Before each experiment, the rings were stretched to an optimal 1 g tension and allowed to equilibrate for 1 h, with washing every 15 min. Changes in the tension were calculated relatively to the baseline tension reach at the end of the equilibration period.

2.3 Protocol

Submaximal contraction was obtained with phenylephrine (PE; 10^{-6} M) in each ring from both groups of rats. Racemic nebivolol, D-nebivolol or L-nebivolol was cumulatively (10^{-6} to 3×10^{-4} M, for each) applied to obtain relaxation in endothelium-intact and endothelium-denuded aortic rings in PE-contracted preparations from both groups. Time intervals between various doses of relaxing drugs were defined as 10 minutes. The endothelium was removed mechanically from some rings by rubbing the intimal surface of the vessels with cotton covered thin glass sticks. Acetylcholine (10^{-5} M) was applied to PE-contracted arteries to determine whether the endothelium was intact. In endothelium-intact arteries, acetylcholine caused approximately 90% relaxation. The absence of the endothelium was confirmed by the loss of relaxation to acetylcholine (to less than 20%).

The relaxation effects of racemic, D-, and L-nebivolol were then obtained in the presence of inhibitors added to the organ chamber 30 min before PE application. The inhibitors were N_ω-nitro-L-arginine methyl ester hydrochloride (L-NAME, a NOS inhibitor; 10^{-3} M) and/or indomethacin (a nonspecific cyclooxygenase inhibitor; 10^{-3} M). Papaverine (10^{-4} M), a smooth muscle relaxing agent, was used to obtain maximum relaxation and to control the relaxation properties of the preparations.

The effect of nebivolol on the PE concentration-response curve was also examined to determine whether nebivolol exhibited α-adrenergic receptor blocking activity because we observed that the aortic rings did not contract in the presence of PE following administration of high doses of nebivolol or its isomers.

2.4 Chemicals

Nebivolol, D-nebivolol, and L-nebivolol (10^{-6} to 3×10^{-4} M) were a gift from Ibrahim Ethem-Menarini (Istanbul, Turkey). Phenylephrine was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetylcholine, papaverine, L-NAME, and indomethacin were also used. Nebivolol, D-nebivolol, and L-nebivolol were dissolved in dimethyl sulfoxide. Indomethacin was dissolved in ethanol. The remaining drugs were dissolved in distilled water.

2.5 Statistical Analysis

Tissue contraction and relaxation responses were recorded as changes under isometric tension in milligrams. The relaxation induced by racemic, D-, and L-nebivolol was expressed as a percentage of the contraction induced by phenylephrine (10^{-6} M).

The findings are shown as mean ± S.E.M. For multiple comparisons between groups, two-way analysis of variance and post hoc Bonferroni tests were applied. Results with P-values less than 0.05 were considered statistically significant.

3. RESULTS

3.1 Nebivolol-induced Relaxation

Phenylephrine-induced contractions were not different in aortic rings derived from rats of 4 and 8 months of age (Fig. 1). Racemic, D- and L-nebivolol (each at 10^{-6} to 3×10^{-4} M) produced concentration-dependent relaxation in the contracted rat arteries (Fig. 2). A significant age-related difference in relaxation was found only for racemic nebivolol and only at the 10^{-4} M concentration in the aortic preparations ($P<0.05$; Fig. 3a). No significant age-related difference was detected for the isomers in the relaxation of the phenylephrine-induced contractions at any concentration (Figs. 3b and 3c). No differences were detected in the responses of the preparations with and without endothelium ($P>0.05$).

3.2 Effect of Inhibitors on the Aortic Relaxation Induced by Racemic, L-, or D-Nebivolol

The NO synthase inhibitor L-NAME (10^{-3} M) alone or with the cyclooxygenase inhibitor indomethacin (10^{-3} M) was added to the organ chamber 30 min before induction of a submaximal phenylephrine contraction. Neither L-NAME alone nor the combined inhibitors

affected the relaxation induced by racemic nebivolol in either age group (Fig. 4a and 4b) or by the isomers (data not shown).

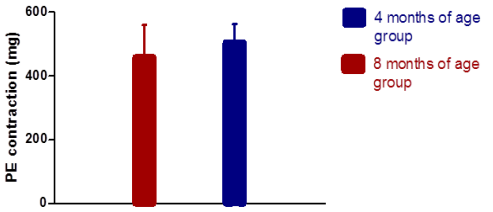

Fig. 1. Phenylephrine (PE)-induced (10^{-6} M) contractions of aortic rings in 4- and 8-month-old rats (n=8)

3.3 Effect of Racemic Nebivolol on the Phenylephrine Concentration-response Curve

In order to investigate whether nebivolol blocked alpha adrenergic receptor activity, various concentrations of racemic nebivolol were examined for their ability to affect the phenylephrine concentration-response curve in the aortic ring preparation. The phenylephrine curve was noncompetitively shifted to the right by three concentrations of nebivolol (10^{-6}, 10^{-5}, and 10^{-4} M; Fig. 5; P>0.05).

The solvents (dimethyl sulfoxide and ethanol) had no effects on vessel contraction and relaxation.

4. DISCUSSION

In the present study, we examined the actions of nebivolol, a relatively new antihypertensive drug with reported beta-1 adrenergic receptor blocking activity, to determine vasodilator responses to this drug in aortic segments obtained from 4- and 8-month-old rats. Nebivolol produces vasodilation in animal and human blood vessels [15,24,25]. The vasodilator effects of nebivolol may be associated with its beta-1 receptor blocking activity, enhanced endothelial NO release [14], beta-2 adrenergic receptor blockade [15], or calcium-activated potassium channel activation [16]. The nebivolol-induced release of no from endothelial cells relaxes vascular smooth muscle [15,19,26,27]. Additionally, It was reported that nebivolol increases NO action as a result of its antioxidant characteristics [26]. Nebivolol also reportedly stimulates beta-3 adrenergic receptors and increases NO release via this pathway [25], beta-3 adrenergic receptors play a role in the relaxation of vascular system [28,29,30].

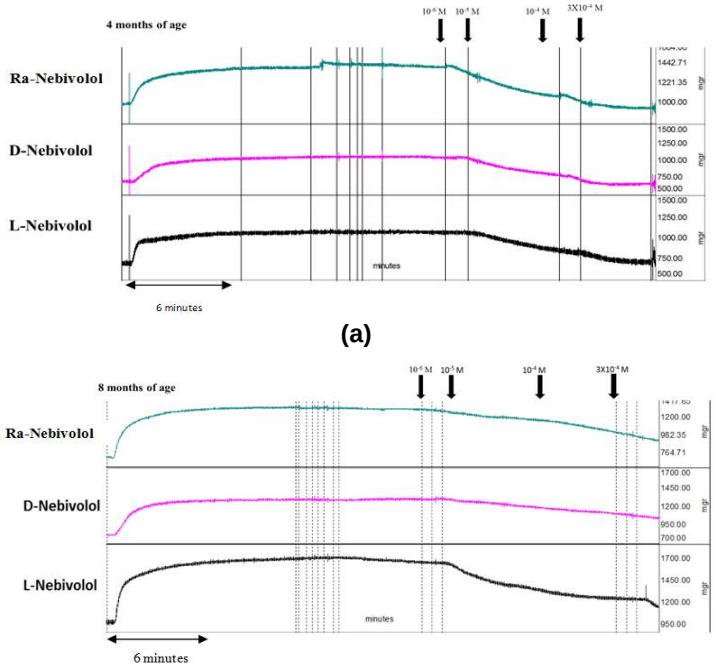

Fig. 2. Representative tracings showing the relaxation of phenylephrine-induced contractions by racemic (Ra), L- and D-nebivolol in aortic rings obtained from rats 4 (a) and 8 (b) months of age (n=8)

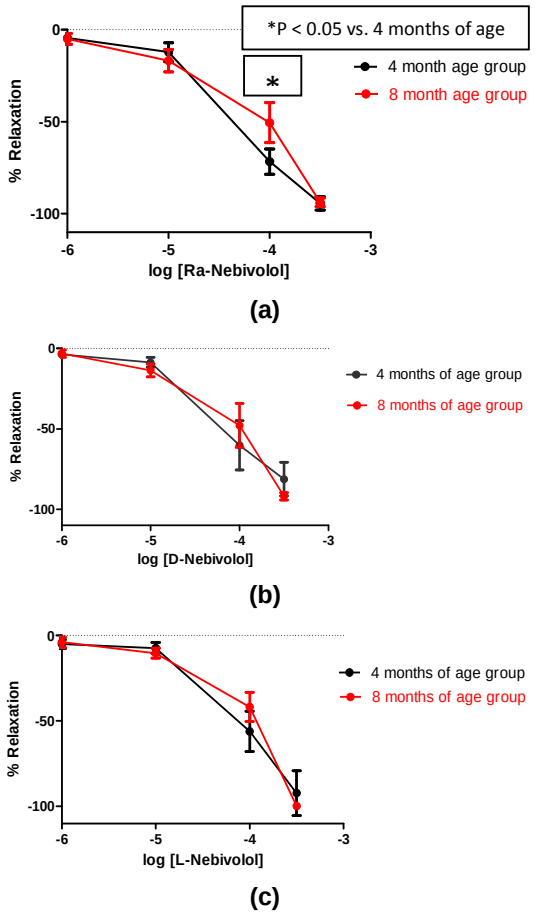

Fig. 3. Age-related differences in relaxation of phenylephrine-induced contractions by racemic (Ra), L- and D-nebivolol in aortic rings obtained from rats 4 and 8 months of age. A significant age-related difference in relaxation was found only for racemic nebivolol and only at a concentration of 10^{-4} M. (n=8)

With age, the presence of some diseases or the dysfunction of the vascular endothelium may change drug effects [10]. Previous studies have reported conflicting age-related results regarding the levels and activity of various agonists. Although a decrease in arterial noradrenaline content was observed in some studies [2-4], no vasoconstrictor responses to noradrenaline were detected with aging [5-8]. By contrast, it was suggested that there was a decrease in both arterial noradrenaline content and sensitivity to exogenous noradrenaline in older individuals [9]. It was reported that there was no influence of age on the noradrenaline response in the presence of an intact endothelium, and responses in denuded preparations appeared to indicate a different role of endothelium on noradrenaline responses in different vascular beds (e.g., rat aorta and tail artery) [21]. It was shown that in endothelium-intact rings, the maximum tension developed by exposure to noradrenaline does not change with age, but the EC_{50} value for noradrenaline increases in the rat aorta [22]. It was suggested that contractile responses to noradrenaline and potassium increase in rat aorta, whereas both of these contractile responses decrease in rabbit aorta with age; however, no change in sensitivity to noradrenaline is observed in rat mesenteric perfused artery [23]. These results show that responses to some drugs may also vary with species and arterial preparations. In contrast to these conflicting reports in the adrenergic system, NO-mediated vasodilation has been shown to decrease in elderly humans, reflecting an age-related decrease in NO synthesis [10-13]. It has been shown that NO-mediated vasodilatation is reduced and total body NO production decreases with age [10,11]. We found no differences in the aortic relaxation responses between 4- and 8-month-old rats. However, this interval (range) between ages might not be suitable to establish the expected differences, and experiments performed with older rats might detect differences in the drug-induced effect, if any, with age. Some drugs are mixtures of stereoisomers with pharmacokinetic characteristics dependent upon one or the other isomer. That is, one stereoisomer can be more responsible than the other for an expected therapeutic effect of the drug [19]. The D-isomer of nebivolol is a more selective beta-1 blocker than the L-isomer [19], whereas the L-isomer produces NO-mediated vasodilation and stimulates eNOS enzyme to a greater degree than the D-isomer [19]. Although we observed relaxation produced by racemic, D-, and L-nebivolol in phenylephrine-contracted rat aortic rings, we did not find any differences among the relaxation responses. Additionally, the responses of the isomers or the racemic mixture did not differ in preparations with and without an intact endothelium. On the other hand, L-NAME, a NOS enzyme inhibitor, and indomethacin,, a cyclooxygenase enzyme inhibitor, had no effect on the relaxation induced by nebivolol. These findings suggest that neither NO nor any other substance derived from the endothelium plays a role in nebivolol-induced relaxation.. Thus, NO and cyclooxygenase metabolites do not play a role in the relaxation induced by racemic nebivolol or its stereoisomers in the rat aorta. However, it has been shown that L-NAME administration blunted vasorelaxation induced by nebivolol on renal artery of mice [16]. This

contrast to the findings could be due to NO-dependency which might change with either animal species or/and vascular segments studied. Taken together, our results indicate that racemic, D-, and L-nebivolol produce relaxation in the same manner and without the release of NO or cyclooxygenase metabolites.

We showed that there was no difference between the two age groups in the contractile response of the aorta to a submaximal concentration of the selective alpha-1 adrenergic receptor agonist PE. Additionally, we showed that PE was unable to contract the aortic rings following administration of high doses of racemic, L-, and D-nebivolol. Therefore, we investigated the effect of nebivolol on the PE concentration-response curve. Nebivolol shifted the PE concentration-response curve noncompetitively to the right. To our knowledge, our group is the second to show an alpha-1 adrenergic receptor blocking effect of nebivolol. It was suggested that nebivolol-induced relaxation results from both inhibition of alpha-1 adrenergic receptors and activation of beta-3 adrenergic receptors in the rat aorta [9]. In our previous report, we presented an alpha-1A adrenergic receptor blocking effect of nebivolol in the rat umbilical artery [31]. The stereoisomers and racemic nebivolol exerted similar effects on vasodilation in the present study, suggesting that they also exert an alpha-1 adrenergic receptor blocking effect in the rat aorta. However, in addition to its inhibition of alpha-1 adrenergic receptor activity, other mechanisms may play a role in the vasodilator effect of nebivolol, such as a calcium channel antagonistic action or membrane stabilizing activity.

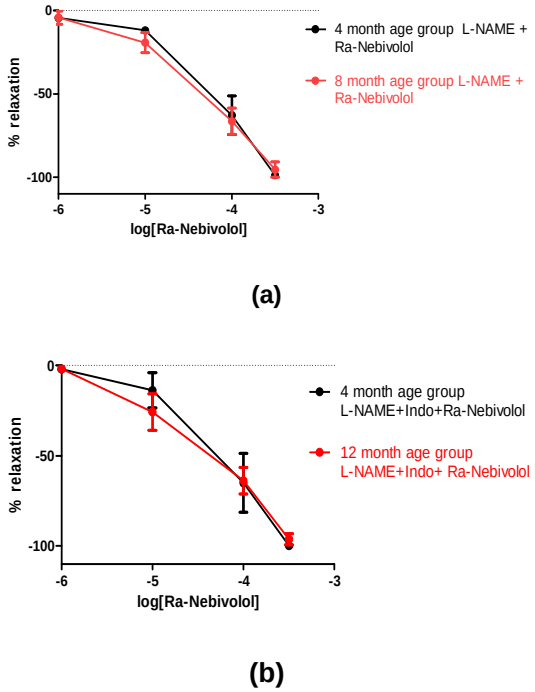

Fig. 4. The inhibitors N_{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME, 10^{-3} M) and indomethacin (10^{-3} M) do not affect the relaxation induced by racemic (Ra) nebivolol in the phenylephrine-contracted aortic rings obtained from rats 4 and 8 months of age (n=8)

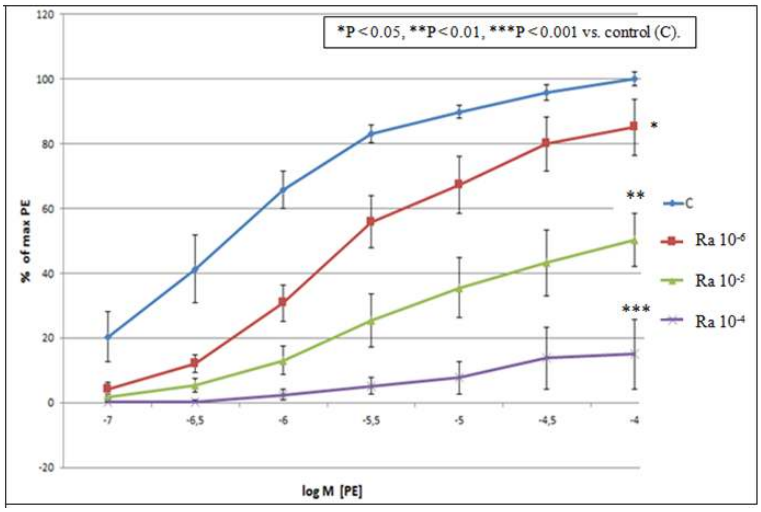

Fig. 5. Effect of racemic nebivolol (Ra, 10^{-6}, 10^{-5}, 10^{-4} M) on the phenylephrine (PE) concentration-response curve in aortic rings obtained from 8-month-old rats (n = 5)

**P < 0.05, **P < 0.01, ***P < 0.001 vs. control (C)*

5. CONCLUSION

The relaxation responses of racemic, D-, and L-nebivolol were similar following phenylephrine-induced contractions in aortic rings obtained from rats 4 and 8 months of age. Whereas cyclooxygenase metabolites and NO did not play a role in the relaxation response, inhibition of the phenylephrine contractions by racemic nebivolol might be due to its alpha adrenergic receptor blocking activity. The same mechanism might be responsible for the relaxation response of the two stereoisomers as their vasodilation response was similar to that of nebivolol. Because the difference in the ages of the rats used in this study was small, future studies with older rats are suggested to show any age-related differences in the effect of nebivolol.

CONSENT

It is not applicable.

ACKNOWLEDGEMENTS

This project was supported by the Baskent University Research Fund. The authors wish to thank Ibrahim Ethem-Menarini in Istanbul, Turkey, for the generous gift of racemic nebivolol and its isomers. We would like to thank Bio. Seda Kursunlu and MsC. Erkan Ermis for their technical support during experimental procedures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Keberle H. Physico-chemical factors of drugs affecting absorption, distribution and excretion. Acta Pharmacol Toxicol (Copenh). 1971;29(suppl 3):30-47.
2. Waterson JG, Frewin DB, Soltys JS. Age-related differences in catecholamine fluorescence of human vascular tissue. Blood Vessels. 1974;11(1-2):79-85.
3. Neubauer B, Christensen NJ. The decrease in noradrenaline concentration in the posterior tibial artery with age. Gerontology. 1978;24(4):299-303.
4. Embree LJ, Roubein IF, Jackson DW, et al. Aging effect on the noradrenaline content of rat brain microvessels. Exp Aging Res. 1981;7(3):215-224.
5. Tuttle RS. Age-related changes in the sensitivity of rat aortic strips to norepinephrine and associated chemical and structural alterations. J Gerontology. 1966;21(4):510-516.
6. Cohen ML, Berkowitz BA. Vascular contraction: Effect of age and extracellular calcium. Blood Vessels. 1976;13(3):139-154.
7. Scott PJ, Reid JL. The effect of age on the responses of human isolated arteries to noradrenaline. Br J Clin Pharmacology. 1982;13(2):237-239.
8. Duckles SP, Carter BJ, Williams CL. Vascular adrenergic neuroeffector function does not decline in aged rats. Circ Res. 1985;56(1):109-116.
9. Fouda AK, Atkinson J. Reduced sensitivity to noradrenaline and electric stimulation of the caudal artery of the aged rat. Arch Mal Coeur Vaiss. 1986;79(6):919-922.
10. Seals DR, Jablonski KL, Donato AJ. Aging and vascular endothelial function in humans. Clin Sci (Lond). 2011;120(9):357-375.
DOI: 10.1042/CS20100476
11. Celermajer DS. Endothelial dysfunction: Does it matter? Is it reversible? J Am Coll Cardiology. 1997;30:325-333.
12. Gerhard M, Roddy M-A, Creager SJ. Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. Hypertension. 1996;27:849-853.
13. Singh N, Prasad S, Singer DR, MacAllister RJ. Ageing is associated with impairment of nitric oxide and prostanoid dilator pathways in the human forearm. Clin Sci. 2002;102:595-600.
14. Altwegg LA, d'Uscio LV, Barandier C, Cosentino F, Yang Z, Luscher TF. Nebivolol induces no-mediated relaxations of rat small mesenteric but not of large elastic arteries. J Cardiovasc Pharmacol. 2000;36:316-320.
15. Broeders MA, Doevendans PA, Bekkers BC, et al. Nebivolol: A third-generation beta-blocker that augments vascular release: Endothelial beta (2)-adrenergic receptor mediated nitric oxide production. Circulation. 2000;102:677-684.
16. Georgescu A, Pluteanu F, Flonta M-L, Badila E, Dorobantu M, Popov D. The cellular mechanisms involved in the vasodilator effect of nebivolol on the renal artery. Eur J Pharmacol. 2005;508:159-166.

17. Rozec B, Quang TT, Noireaud J, Gauthier C. Mixed beta 3-adrenoceptor agonist and alpha 1-adrenoceptor antagonist properties of nebivolol in rat thoracic aorta. Br J Pharmacol. 2006;147:699-706.
18. Wang Y, Zhang M, Liu Y, Li J, Song E, Niu L, Cheng N. Neither K^+ channels nor PI3K/Akt mediates the vasodilative effect of nebivolol on different types of rat arteries. J Cardiovasc Pharmacol Ther. 2009;14(4):332-8.
19. Evangelista S, Garbin U, Pasini AF, Stranieri C, Boccioletti V, Cominacini L. Effect of dl-nebivolol, its enantiomers and metabolites on the intracellular production of superoxide and nitric oxide in human endothelial cells. Pharmacol Res. 2007;55: 303-309.
20. Rees DD, Palmer RM, Hodson HF, Moncada S. A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. Br J Pharmacol. 1989;96(2):418-424.
21. Tabernero A, Vila E. Effect of age on noradrenaline responses in rat tail artery and aorta: Role of endothelium. J Auton Pharmacol. 1995;15(5):327-333.
22. Hashimoto M, Gamoh S, Hossain S, et al. Age-related changes in aortic sensitivity to noradrenaline and acetylcholine in rats. Clin Exp Pharmacol Physiol. 1998;25(9): 676-681.
23. Carvajal A, Velasco A, Martín Arias LH, Maroto M. Age-related changes in contractile responses to noradrenaline in isolated blood vessels from rat and rabbit. J Auton Pharmacol. 1995;15(5):321-326.
24. Ritter JM. Nebivolol: Endothelium-mediated vasodilating effect. J Cardiovasc Pharmacol. 2001;38:(suppl)13-16.
25. de Groot AA, Mathy MJ, Van Zwieten PA, Peters SL. Involvement of the beta3 adrenoceptor in nebivolol-induced vasorelaxation in the rat aorta. J Cardiovasc Pharmacol. 2003;42:232-236.
26. Kakoki M, Hirata Y, Hayakawa H, et al. Effects of vasodilatory beta-adrenoceptor antagonists on endothelium-derived nitric oxide release in rat kidney. Hypertension. 1999;33:467-471.
27. Ladage D, Brixius K, Hoyer H, et al. Mechanisms underlying nebivolol-induced endothelial nitric oxide synthase activation in human umbilical vein endothelial cells. Clin Exp Pharmacol Physiol. 2006;33:720-724.
28. Trochu N, Leblais V, Rautureau Y, et al. Beta 3-adrenoceptor stimulation induces vasorelaxation mediated essentially by endothelium-derived nitric oxide in rat thoracic aorta. Br J Pharmacol. 1999;128: 69-76.
29. Oelze M, Daiber A, Brandes RP, et al. Nebivolol inhibits superoxide formation by NADPH oxidase and endothelial dysfunction in angiotensin II-treated rats. Hypertension. 2006;48:677-684.
30. Rautureau Y, Toumaniantz G, Serpillon S, Jourdon P, Trochu J-N, Gauthier C. Beta 3-adrenoceptor in rat aorta: Molecular and biochemical characterization and signalling pathway. Br J Pharmacol. 2002;137:153-161.
31. Guler S, Cekic EG, Tuncer M. An α-adrenergic receptor blocking effect of nebivolol in rat uterine artery. 20th Turkish Pharmacology Congress, Manavgat Antalya, Turkey. 2009;268-87.
Available: www.tfd2009.kongresi.info

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/16867