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Beta adrenergic receptors play role in the vasoconstrictor effect of dobutamine in the isolated rat aorta

İzole sıçan aortunda dobutaminin vazokonstriktör etkisinde beta adrenerjik reseptörler rol oynar

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Abstract

Purpose: Experimental evidence exists that cardiac action of dobutamine is mediated by alfa-1 and beta-adrenergic receptors. However, uncertainty remains regarding the vascular effect of dobutamine and contribution of beta-adrenergic receptors to this effect. The aim of the present study was to investigate the direct effect of dobutamine in the rat aorta and the role of beta-adrenergic receptors in this effect.

Materials and methods: The isolated thoracic aortic rings were mounted in organ bath containing Krebs-Henseleit solution. After an equilibration period, endothelial integrity was then checked by the response to acetylcholine (10 μM) in aortic rings pre-contracted with phenylephrine (1 μM). After washout, dobutamine (0.001-10 μM) was added to generate cumulative concentration–response curves (CCRCs). To investigate the role of alfa- and beta- adrenergic receptors in the dobutamine-induced vascular response, prazosin (0.0003 μM) or propranolol (1 μM) was added to the bath medium 30 min before the addition of dobutamine in some experiments.

Results: Dobutamine produced concentration-dependent contraction in the endothelium-intact isolated rat aorta. This effect was significantly inhibited by either propranolol or prazosin (p<0.05). Prazosin also significantly supressed the maximum vascular response obtained by dobutamine (p<0.05).

Conclusion: The results, to the best of my knowledge, demonstrates for the first time that beta-adrenergic receptors are involved in the vasoconstrictor effect of dobutamine in the endothelium-intact rat aorta.

Key words: Dobutamine, alfa adrenergic receptor, beta adrenergic receptor, vasoconstriction, aorta.

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Özet

Amaç: Alfa-1 ve beta-adrenerjik reseptörlerin, dobutaminin kardiyak etkisine aracılık ettiğine ilişkin deneysel kanıt mevcuttur. Ancak, dobutaminin vasküler etkisi ve bu etkiye beta-adrenerjik reseptörlerin katkısı belirsizliğini korumaktadır. Bu çalışma, dobutaminin sıçan aortundaki doğrudan etkisini ve bu etkide beta adrenerjik reseptörlerin rolünü araştırmayı amaçlamıştır**.**

Gereç ve yöntem: İzole edilmiş torasik aort halkaları Krebs-Henseleit solüsyonu içeren organ banyosuna asıldı. Dinlenme periyodu sonrası, aort halkaları fenilefrinle (1 μM) kasıldıktan sonra verilen asetilkolin (10 μM) yanıtıyla endotelyal bütünlük kontrol edildi. Yıkama sonrası, dobutamin (0,001-10 μM), kümülatif konsantrasyon-yanıt eğrilerini elde etmek için organ banyosuna eklendi. Dobutaminin oluşturduğu vasküler yanıtta alfa- ve beta-adrenerjik reseptörlerin rolünü araştırmak için, bazı deneylerde, prazosin (0,0003 μM) veya propranolol (1 μM) banyo ortamına dobutaminden 30 dakika önce eklendi.

Bulgular: Dobutamin, endoteli sağlam izole sıçan aortunda konsantrasyon-bağımlı kontraksiyona neden oldu. Bu etki, propranolol veya prazosin varlığında anlamlı olarak inhibe edildi (p<0,05). Ayrıca, prazosin dobutaminle elde edilen maksimum vasküler yanıtı da baskıladı (p<0,05).

Sonuç: Bu sonuçlar, bildiğim kadarıyla, ilk defa endoteli sağlam izole sıçan aortunda dobutaminin vazokonstriktör etkisinde beta-adrenerjik reseptörlerin ilişkisi olduğunu göstermektedir.

Anahtar kelimeler: Dobutamin, alfa adrenerjik reseptör, beta adrenerjik reseptör, vasokontsriksiyon, aort.

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Introduction

Dobutamine has a therapeutic value as an inotropic agent more than 40 years. It is used as a racemic mixture of two stereoisomers. Therefore, pharmacological and clinical data regarding the effects of dobutamine are primarily based on the racemate.

The inotropic response elicited by dobutamine was initially attributed largely to its agonistic action on myocardial beta-1(β_1)-adrenergic receptors (ARs) [1]. Later, it was shown that agonistic efficacy of dobutamine on alfa-1 (α_1)-ARs also plays role in this inotropic response [2, 3]. The agonistic effect of dobutamine on α_1- ARs has also been investigated in different vascular beds as the important role of these receptors in the vasoconstrictor response [4-6]. Taken together, these experiments indicate that dobutamine's positive inotropic and vasoconstrictor effects share a common underlying mechanism: stimulation of α_1- ARs.

In most studies up to date, the vascular experiments were mainly designed using dobutamine in precontracted arteries [7-9]. However, the direct effect of dobutamine on vascular response and contribution of β- ARs to this response still remain unclear.

Therefore, the aim of the present study was to investigate the direct effect of dobutamine in the rat aorta with intact endothelium and the involvement of β- ARs in this effect.

Materials and methods

Drugs and chemicals

Phenylephrine hydrochloride (PE), acetylcholine hydrochloride (ACh), Dobutamine hydrochloride, prazosin hydrochloride and propranolol hydrochloride were obtained from Sigma-Aldrich (USA).

Animals

The experimental procedures were approved by the Ethical Committee for Animal Studies and conformed to the guidelines proposed in the Guide for the Care and Use of Laboratory Animals. 250-300 g male Sprague–Dawley rats were used in this study. The rats were allowed to have free access to food and tap water.

Experimental protocol

The anesthetized rats were sacrificed by cervical dislocation. Afterwards, the descending thoracic aorta was rapidly dissected out and placed in Krebs-Henseleit solution (KHS) composed of (mM): NaCl, 118; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; and glucose, 11). The thoracic aorta was carefully cleaned of surrounding fat and connective tissue and cut into aortic rings approximately 3 mm in length. The aortic rings were mounted between two stainless hooks in 10 ml organ baths containing KHS (at 37 °C bubbled with 95% O_2 +5% CO_2) and attached to force displacement that were connected to data acquisition system (Biopac, MP30, CA, USA) The aortic rings were placed under 2 g resting tension and allowed to equilibrate for 60 min with washing fresh KHS every 15 min.

After the equilibration period, the integrity of the vascular endothelium was checked by contracting the tissues with submaximal PE (1 μM) and adding ACh (10 μM). Only tissues that relaxed by more than 50% to ACh were included in this study. Dobutamine (0.001–10 μM) was cumulatively added to organ bath and obtained cumulative concentration response curves (CCRCs) of the endothelium-intact aortic rings (n=6). In order to elucidate the impact of β-ARs in this effect, propranolol (non-selective β-AR antagonist, 1 μM) was added to the bath medium 30 min before the addition of dobutamine in some experiments (n=5). Additionally, the direct effect of dobutamine was also evaluated in the aortic rings incubated with prazosin (α_1- AR antagonist, 0.0003 μM) for 30 min (n=4).

Statistical analysis

Data are expressed as mean±*SEM.* Contractile effect of dobutamine was expressed as percentage of the contraction induced by phenylephrine (1 μM). All statistical analyses were performed with using the statistical software (GraphPad Prism, USA). Multiple comparisons were performed using ANOVA followed by post-hoc Bonferroni test. Efficacy of dobutamine was

expressed as maximum contraction (E_{max}) to dobutamine. The pD_2 values were calculated for potency of dobutamine, which is the negative logarithm of the half maximum effective concentration (EC_{50}). $p<0.05$ was considered to be statistically significant.

Results

Dobutamine produced concentration-dependent contraction in the endothelium-intact aortic rings (Fig 1A, Fig 1B (n=6)). The vasoconstrictor effect of dobutamine at increasing concentrations was significantly inhibited by the presence of propranolol (Fig 1B, $p<0.05$, n=5). Additionally, incubation of aortic rings with propranolol caused rightward shift without significant reduction in the E_{max} (Fig 1B, Fig 2). Similarly, prazosin significantly inhibited the CCRC of dobutamine but also supressed the maximum response of dobutamine (Fig 2, $p<0.05$, n=4).

Analyzing the CCRCs, the potency of dobutamine as attested by pD_2 value was significantly reduced by the incubation with propranolol or prazosin (Fig 3, $p<0.05$).

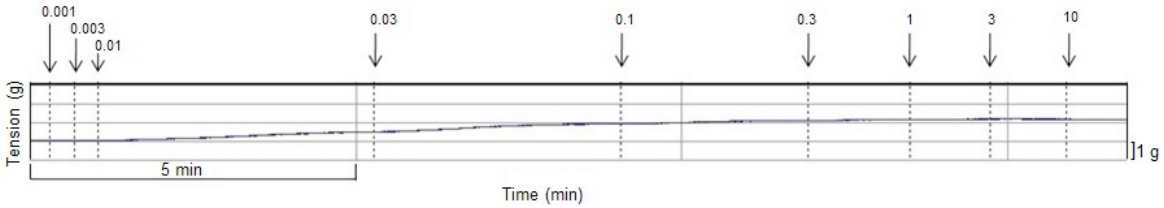

Figure 1A. Orginal trace of contractile effect of dobutamine in the endothelium-intact aorta. Arrows indicate addition of dobutamine and values are concentration (μM)

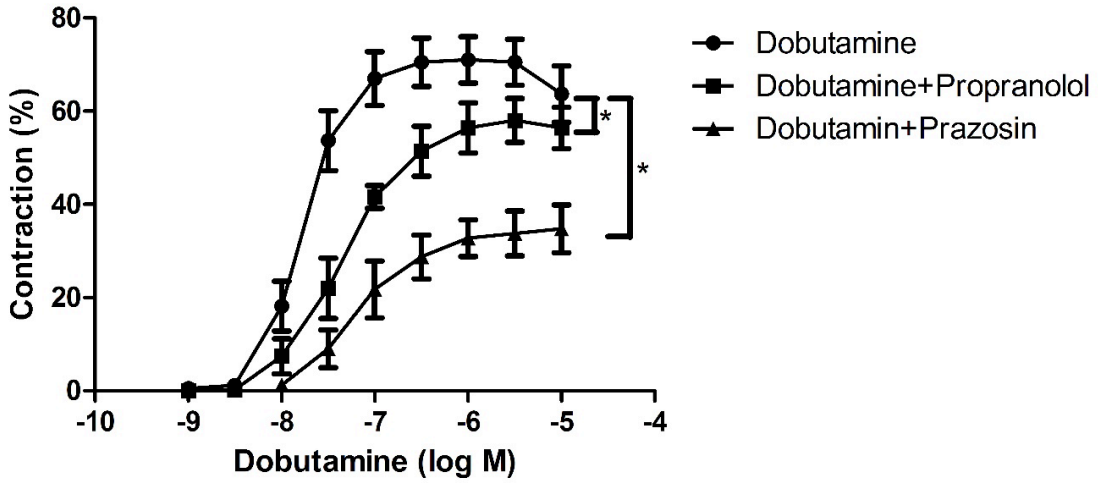

Figure 1B. Cumulative concentration-response curves of dobutamine (10^{-9}-10^{-5} M) in the absence (n=6) and presence of propranolol (non-selective β-AR antagonist, 1 μM, n=5) or prazosin (α_1-adrenoceptor antagonist, $3x10^{-4}$ μM, n=4) in the endothelium-intact aortas. *$p<0.05$ vs Dobutamine

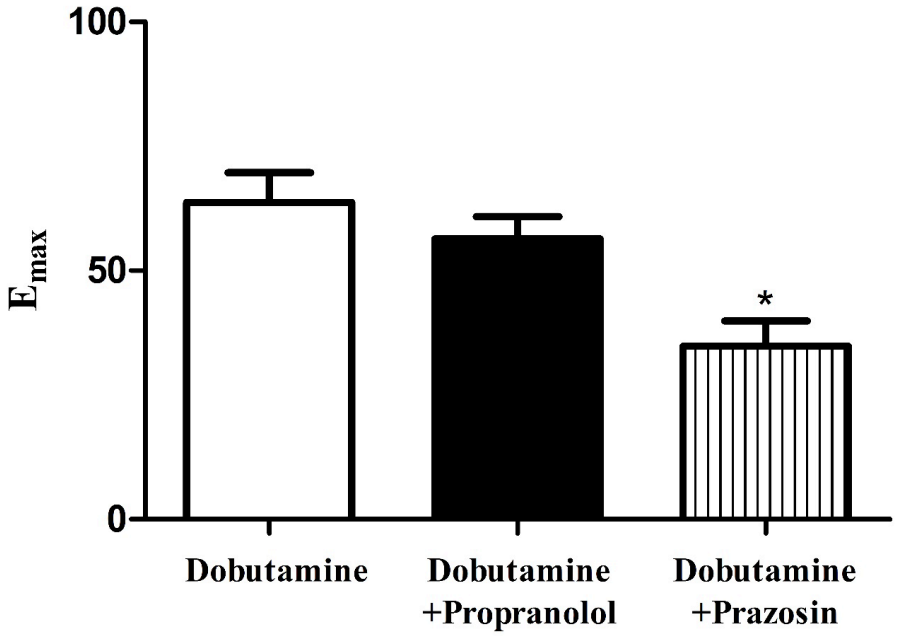

Figure 2. E_{max} to dobutamine in the absence (n=6) and presence of propranolol (non-selective β-AR antagonist, 1 μM, n=5) or prazosin (α1-adrenoceptor antagonist, 3x10-4 μM, n=4) in the endothelium-intact aortas. *p<0.05 vs Dobutamine

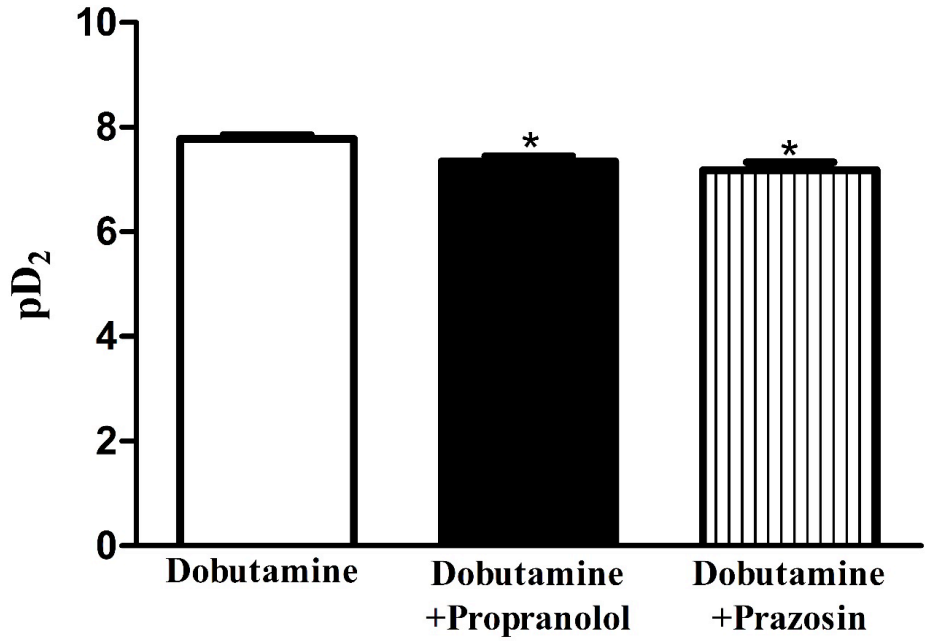

Figure 3. pD_2 of dobutamine in the absence (n=6) and presence of propranolol (non-selective β-AR antagonist, 1 μM, n=5) or prazosin (α1-adrenoceptor antagonist, 3x10-4 μM, n=4) in the endothelium-intact aortas. *p<0.05 vs Dobutamine

Discussion

The results of the present study showed that dobutamine produced concentration-dependent contraction in the endothelium-intact rat aorta. Findings from the present study also demonstrated for the first time, to the best of my knowledge, the β-ARs are involved in the vasoconstrictor effect of dobutamine in the rat aorta.

Dobutamine is a chiral molecule with two stereoisomers including (+)- and (-)-dobutamine [10]. Previous evidence has indicated that each stereoisomer of dobutamine represents different agonistic activity on α_1- and β_1-adrenergic receptor subtypes [4, 11]. However, dobutamine is clinically used as a racemic mixture and so overall pharmacologic response results from the net effect of these two stereoisomers. Therefore, the present study was designed to investigate the direct action of dobutamine by using its racemate.

Although knowledge of the vasorelaxant action of dobutamine is based on the previous studies applying dobutamine in precontracted arteries [7-9], the direct vascular action of dobutamine is also important. Therefore, the experiments described in this present study were designed to elucidate the direct action of dobutamine in the isolated aorta. As a result, present findings indicate that dobutamine contracts the endothelium-intact aorta in a concentration-dependent way. These results are in line with other authors, who also reported that dobutamine elicited contraction in the various isolated vessels including aorta, renal, mesenteric and femoral arteries [4, 5, 12, 13]. Interestingly, Ozaki et al. [12] showed that the contractile response of dobutamine at increasing concentrations ($5x10^{-8}$-10^{-4} M) in renal and mesenteric arteries was converted to relaxation in the presence of phenoxybenzamine indicating the role of α-ARs in this contractile response [12]. In contrast, the vasodilatory response of coronary and cerebral arteries was obtained with the same concentrations of dobutamine [12]. Accordingly, these different responses are thought to be involved in the agonistic activity of dobutamine at both α- and β-ARs and relative functional importance of these receptors in the control of vascular response of different arteries [14]. These contradictory results are also present under in vivo experiments showing increased and decreased mean arterial pressure with dobutamine at lower doses (4-8 mg/kg,) and higher doses (16-32 mg/kg), respectively [15]. Furthermore, it has been found that dobutamine didn't cause any vasocontractile response in internal thoracic artery segments from patients undergoing coronary bypass surgery [16]. This evidence also indicates that the vascular response elicited by dobutamine could be modified in the presence of cardiovascular diseases.

In the present study, it has been shown that the vasocontractile response produced by dobutamine is based on its agonistic activity on α- and β-ARs. A partial agonistic activitity of dobutamine on a-adrenoceptors has previously been reported [2, 17]. This may also help to explain the difference in direct and indirect vascular actions of dobutamine.

Dobutamine has been reported to have selectivity on different β-adrenergic receptor subtypes involving β_1, β_2 and β_3 [18]. These receptors are also present in vasculature [19]. However, the relevance of β-adrenergic receptors in the vascular response to dobutamine has not been questioned. Therefore, the novel aspect of the present study is the role of β-adrenergic receptors in the dobutamine-mediated vascular response of the endothelium-intact aorta. In this regard, a main evidence from the present study is that propranolol, non-selective b-AR antagonist, inhibited dobutamine-induced vasoconstriction with no significant changes in the efficacy. Furthermore, present findings confirm involvement of the α_1-adrenergic receptors in this response to dobutamine as reported previously [5, 6]. Additionally, the efficacy of dobutamine has been found to be decreased by the presence of prazosin in the present study.

The involvement of the endothelium in the vascular homeostasis has been widely documented [20]. In this regard, there is increasing evidence showing that endothelial β-ARs especially β_2- and β_3-ARs are primarily responsible for the vasorelaxant response [7, 8, 21, 22]. Although endothelial β-ARs have been found to be responsible for the vasocontractile response to dobutamine in the present study, the contribution of each β-ARs in this response needs to be further investigated.

In conclusion, the results of the present study, to the best of my knowledge, demonstrate for the first time β-ARs are also involved in the contractile effect of dobutamine in the rat aorta. Taken together, the present findings reveal a pharmacological action elicited by activation of α_1- and β-ARs in the vasocontractile effect of dobutamine.

Conflict of interest: No conflict of interest was declared by the authors.

References

1. Tuttle RR, Mills J. Dobutamine: development of new catecholamine to selectively increase cardiac contractility. Circ Res 1975;36:185-196. https://doi.org/10.1161/01.res.36.1.185
2. Kenakin TP. An in vitro quantitative analysis of the alpha adrenoceptor partial agonist activity of dobutamine and its relevance to inotropic selectivity. J Pharmacol Exp Ther 1981;216:210-219.
3. Kenakin TP, Johnson SF. The importance of the alpha-adrenoceptor agonist activity of dobutamine to inotropic selectivity in the anaesthetized cat. Eur J Pharmacol 1985;111:347-354. https://doi.org/10.1016/0014-2999(85)90641-7
4. Ruffolo RR, Spradlin TA, Pollock GD, Waddell JE, Murphy PJ. Alpha and beta adrenergic effects of the stereoisomers of dobutamine. J Pharmacol Exp Ther 1981;219:447-452.
5. Oriowo MA, Nichols AJ, Ruffolo RR. Receptor protection studies with phenoxybenzamine indicate that a single alpha 1-adrenoceptor may be coupled to two signal transduction processes in vascular smooth muscle. Pharmacology 1992;45:17-26. https://doi.org/10.1159/000138968
6. Oriowo MA. Alpha1-adrenoceptor antagonist effect of (+/-)- dobutamine in rat isolated gastric artery preparation. J Cardiovasc Pharmacol 1999;34:728-733. https://doi.org/10.1097/00005344-199911000-00015
7. Flacco N, Segura V, Perez Aso M, et al. Different β-adrenoceptor subtypes coupling to cAMP or NO/cGMP pathways: implications in the relaxant response of rat conductance and resistance vessels. Br J Pharmacol 2013;169:413-425. https://doi.org/10.1111/bph.12121
8. Pourageaud F, Leblais V, Bellance N, Marthan R, Muller B. Role of beta2 adrenoceptors (beta-AR), but not beta1-, beta3-AR and endothelial nitric oxide, in beta-AR-mediated relaxation of rat intrapulmonary artery. Naunyn Schmiedebergs Arch Pharmacol 2005;372:14-23. https://doi.org/10.1007/s00210-005-1082-2
9. Randall MD, McCulloch AI. The involvement of ATP-sensitive potassium channels in beta-adrenoceptor-mediated vasorelaxation in the rat isolated mesenteric arterial bed. Br J Pharmacol 1995;115:607-612. https://doi.org/10.1111/j.1476-5381.1995.tb14975.x
10. Waldeck B. The β1-selective adrenoceptor agonist dobutamine: a fallacy being perpetuated. Chirality 2011;23:63-64. https://doi.org/10.1002/chir.20874
11. Ruffolo RR, Yaden EL. Vascular effects of the stereoisomers of dobutamine. J Pharmacol Exp Ther 1983;224:46-50.
12. Ozaki N, Kawakita S, Toda N. Effects of dobutamine on isolated canine cerebral, coronary, mesenteric, and renal arteries. Cardiovasc Pharmacol 1982;4:456-461. https://doi.org/10.1097/00005344-198205000-00017
13. Morishita H, Furukawa T. Possible modes of action of dobutamine in dog femoral and pulmonary arteries. Cardiovasc Res 1980;14:103-107. https://doi.org/10.1093/cvr/14.2.103
14. Kenakin TP, Johnson SF. The importance of the alpha-adrenoceptor agonist activity of dobutamine to inotropic selectivity in the anaesthetized cat. European J of Pharmacol 1985;111:347-354. https://doi.org/10.1016/0014-2999(85)90641-7
15. Robie NW, Nutter DO, Moody C, McNay JL. In Vivo Analysis of Adrenergic Receptor Activity of Dobutamine. Circ Res 1974;34:663-671. https://doi.org/10.1161/01.res.34.5.663
16. Myers ML, Li GH, Yaghi A, McCormack D. Human internal thoracic artery reactivity to dopaminergic agents. Circulation 1993;88:110-114.
17. Kenakin, TP. Organ selectivity of drugs. Alternatives to receptor selectivity, Trends in Pharmacol Sci 1982;3:153-156. https://doi.org/10.1016/0165-6147(82)91067-7
18. Baker JG. The selectivity of beta-adrenoceptor agonists at human beta1-, beta2- and beta3-adrenoceptors. Br J Pharmacol 2010;160:1048-1061. https://doi.org/10.1111/j.1476-5381.2010.00754.x
19. Werstiuk ES, Lee RM. Vascular beta-adrenoceptor function in hypertension and in ageing. Can J Physiol Pharmacol 2000;78:433-452.
20. Félétou M, Vanhoutte PM. Endothelial dysfunction: a multifaceted disorder. Am J Physiol Heart Circ Physiol 2006;291:985-1002. https://doi.org/10.1152/ajpheart.00292.2006
21. Mori A, Miwa T, Sakamoto K, Nakahara T, Ishii K. Pharmacological evidence for the presence of functional beta(3)-adrenoceptors in rat retinal blood vessels. Naunyn Schmiedebergs Arch Pharmacol 2010;382:119-126. https://doi.org/10.1007/s00210-010-0526-5