NO-DEPENDENT VASODILATION INDUCED BY NEBIVOLOL IN CORONARY CIRCULATION IS NOT MEDIATED BY β-ADRENOCEPTORS OR BY 5 HT₁A-RECEPTORS

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INTRODUCTION

Nebivolol is a unique β₁-adrenoceptor antagonist which possesses peripheral vasodilator properties dependent on endothelial NO. Nebivolol-induced release of NO was attributed to its L stereoisomer and to its ability to stimulate endothelial β₂, β₃ adrenoceptors or 5-HT₁A receptors. Here, in the isolated guinea pig heart we analysed coronary vasodilator potency of L- and D-nebivolol and a possible role of β₂, β₃ adrenoceptors and 5-HT₁A receptors in nebivolol-induced vasodilation. Surprisingly, we found that not only L-nebivolol (3-30x10⁻⁶ M) but also D-nebivolol (3-30x10⁻⁶ M) induced coronary vasodilation, and both responses were inhibited by L-NAME (10⁻⁴ M). In contrast with the stereoisomers of nebivolol, atenolol at the equimolar concentrations did induce slight vasoconstriction. The nonselective β₁/β₂-adrenoceptor antagonist - nadolol (10⁻⁴ M), the selective β₂-adrenoceptor antagonist - L 748337 (10⁻⁴ M) and the 5-HT₁A receptor antagonist - NAN 190 (5 x 10⁻⁶ M), none of them inhibited coronary vasodilation induced by D- and L-nebivolol. In summary, in the isolated guinea pig heart both D- and L-nebivolol act as coronary vasodilators. Coronary vasodilation induced by stereoisomers of nebivolol is mediated by endothelium-derived NO and does not depend on β₂, β₃ adrenoceptors or 5 HT₁A receptors.
Vasodilator action mediated by endothelial nitric oxide (NO) (1-3). Importantly, endothelial action of nebivolol has recently gained clinical significance, as it was shown that treatment of hypertension with nebivolol, but not with atenolol, was associated with the reversal of endothelial dysfunction (4).

Interestingly, nebivolol is a racemic mixture of D- and L-stereoisomers and it is thought that each stereoisomer has distinct pharmacological properties. D-nebivolol is considered to be a selective β1-adrenoceptor antagonist, while L-nebivolol is claimed to have peripheral vasodilator properties (5,6).

Vasodilator action of nebivolol was reported many times to be mediated by endothelium-derived NO (1-3,7-9). Yet, the mechanism by which nebivolol releases NO from endothelium remains unclear. Not long ago it was proposed that endothelial β2, β3-adrenoceptors or 5HT1A receptors might be involved (9-11).

The aim of the present study was to assess a possible contribution of β-adrenoceptors as well as 5HT1A receptors to nebivolol-induced vasodilation in the isolated guinea pig heart. We analysed coronary vasodilator potency of D and L stereoisomers of nebivolol. To assess the involvement of endothelial NO in nebivolol-induced vasodilation we used the non-selective NOS inhibitor - L-NAME (10−4 M). For assessment of the involvement of β-adrenoceptors and 5HT1A receptors appropriate receptor antagonists were used.

**MATERIAL AND METHODS**

**Langendorff preparation of guinea pig heart**

The details of the method were described elsewhere (12). The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health, and the experimental procedures used in the present study were approved by the local Animal Research Committee.

Briefly, guinea pigs of both sex and body weight of 300 - 400 g were anaesthetised with pentobarbital (30 - 40 mg kg−1 body weight). Their hearts were isolated, washed in ice cold saline, and mounted in Langendorff apparatus of Hugo Sachs Electronics (HSE). Guinea pig hearts were perfused retrogradely through aorta under a constant perfusion pressure of 60 mm Hg with Krebs-Henseleit buffer of the following composition (mM): NaCl 118, CaCl2 2.52, MgSO4 1.64, NaHCO3 24.88, K2HPO4 1.18, glucose 5.55, sodium pyruvate 2.0, equilibrated with 95%O2 + 5% CO2 at 37°C in the oxygenator with rotating disc (HSE). The hearts were paced with 273 impulses per min through two platinum electrodes placed in the right atrium. Left ventricular pressure (LVP) was measured using the fluid-filled balloon inserted into the left ventricle and connected to a pressure transducer (Isotec HSE). The end diastolic pressure was adjusted to be less than 10 mm Hg. The dP/dtmax and dP/dtmin values were calculated from LVP signal by an analogue differentiation amplifier (DIF module HSE). Coronary flow (CF) was monitored by Ultrasonic flowmeter (HSE). LVP, dP/dtmax, dP/dtmin and CF were calibrated once a day before the experiment and then continuously displayed throughout the experiment and finally analysed using the specially-designed software (PSCF. EXE-IGEL, Poland).
**Protocol of experiments**

For studying coronary vasodilator response either D-nebivolol or L-nebivolol were given as 1 min intracoronary infusion at final concentrations of \(10^{-6} - 3 \times 10^{-4}\) M. D-nebivolol or L-nebivolol were infused twice: in the absence and in the presence of an inhibitor. In control experiments, in the absence of inhibitors, coronary vasodilator responses to stereoisomers of nebivolol were highly reproducible (data not shown).

The involvement of endothelium-derived NO in coronary vasodilator responses was assessed by pretreatment with L-N\(^{G}\)-nitroarginine methyl ester (L-NAME, \(10^{-4}\) M). On the other hand, contribution of \(\beta\) adrenoceptors to the coronary vasodilation induced by D- and L-nebivolol was assessed by \(\beta_3/\beta_2\)-adrenoceptor antagonist nadolol (\(10^{-4}\) M) and \(\beta_2\) adrenoceptor antagonist L-748337 (\(10^{-4}\) M). The participation of 5 HT\(_{1A}\) receptors in these responses were studied with 5 HT\(_{1A}\) receptor antagonist NAN 190 (\(5 \times 10^{-6}\) M). All inhibitors were infused for at least 15 min before eliciting a response to nebivolol.

Both stereoisomers of nebivolol were dissolved in the mixture of DMSO and water (v/v 1:1) or in DMSO alone (when used at the highest concentration of \(3 \times 10^{-4}\) M) and infused into the coronary circulation at a rate of 0.03 - 0.09 ml min\(^{-1}\). The rate of infusion was adjusted to the value of basal coronary flow. Infusion of DMSO alone or DMSO + H\(_2\)O slightly increased coronary flow by 1.20 ± 0.18 ml/min and 1.07 ± 0.16 ml/min, respectively.

Duration of an experiment never exceeded three hours, up to this period of time quality of preparation of isolated guinea pig heart stayed mostly unchanged.

**Statistical methods**

All values were expressed as means ± SEM. Statistical significance was evaluated by the unpaired t-test. A p value less than 0.05 was considered to be significant.

**RESULTS**

In the isolated guinea pig heart basal level of coronary flow was \(9.54 ± 0.41\) ml min\(^{-1}\). Both D- and L-nebivolol, in micromolar range of concentrations, increased coronary flow in a concentration-dependent manner (Fig. 1A, 1B). Magnitude of coronary flow responses to D-nebivolol and L-nebivolol was similar. An increase in coronary flow induced by L-nebivolol or D-nebivolol at a concentration of \(3 \times 10^{-4}\) M mounted up to 10.97 ± 0.69 ml/min and 10.03 ± 0.60 ml/min, respectively. Atenolol at the same range of concentrations did not cause vasodilation but slightly reduced coronary flow (Fig. 1A).

In the presence of NOS inhibitor, L-NAME (\(10^{-4}\) M) coronary vasodilator responses to D- and L-nebivolol were substantially inhibited (Fig. 2).

Neither the nonselective \(\beta_1\) and \(\beta_2\)-adrenoceptor antagonist, nadolol (\(10^{-5}\) M), nor the selective \(\beta_2\)-adrenoceptor antagonist, L-748337 (\(10^{-4}\) M) did influence coronary vasodilator response to D- nebivolol or L-nebivolol (Fig. 3, 4). Also HT\(_{1A}\) receptor antagonist NAN 190 (\(10^{-6}\) M) did not change these responses (Fig. 5A).

As compared to nebivolol, coronary flow responses induced by serotonin were smaller and had different characteristics (Fig. 5A, 5B). Firstly, serotonin-induced
Fig. 1. A. Effects of D-nebivolol, L-nebivolol and atenolol on coronary flow in the isolated guinea pig heart (concentration-dependent curves). Data are expressed as an increase in coronary flow (ml min⁻¹). Results are mean±SEM (n = 7 - 10). B. Original tracing of an experiment showing coronary flow response to D-nebivolol, L-nebivolol and D,L-nebivolol. Coronary flow responses to bradykinin (Bk) or reactive hyperemia (RH) evoked by a 15 sec coronary occlusion are shown for comparison.
vasodilation was blunted by NAN 190. Secondly, kinetics of coronary flow increase in response to serotonin was sluggish as compared to instant vascular response induced by D- or L-nebivolol (Fig. 5A, 5B).

DISCUSSION

Here we demonstrate that in the isolated guinea pig heart both D-nebivolol and L-nebivolol are equally potent in their ability to induce NO-dependent vasodilation. We also provide evidence that nebivolol-induced coronary vasodilation is not mediated either by β-adrenoceptors or by 5 HT_{1A} receptors.

The ability of nebivolol to induce NO-dependent vasodilation, shown here, is not a new finding. Indeed, involvement of endothelial NO in vasodilator action of racemic nebivolol was previously reported in peripheral vessels of animals (2,3) and humans (1,7,13), as well as in the coronary circulation of isolated guinea pig heart (14).

However, our finding that both stereoisomers of nebivolol induce vasodilation may seem to contradict the results of previous studies. It is generally accepted, mainly on the basis of in vivo studies, that β-adrenoceptor antagonistic properties are confined to D-nebivolol while peripheral vasodilator action to L-nebivolol (3,5,6,15). In the isolated guinea pig heart D-nebivolol, but not L-nebivolol (10^{-6} M) inhibited isoprenaline-induced increase in contractility (data not shown). Thus, we agree that D-nebivolol but not L-nebivolol display β-adrenoceptor antagonistic properties. However, in our hands, vasodilator effect of nebivolol was not confined to its L-stereoisomer as both D- and L-nebivolol were equipotent as coronary vasodilators.
One reason for this discrepancy might be that *in vivo* vasodilator effect of D-nebivolol is blunted by vasoconstrictor reflex induced by a decrease in heart contractility subsequent to β-adrenoceptor blockade of D-nebivolol (5,6,15). On the other hand, it seems likely that the lack of stereoselectivity in nebivolol-induced coronary vasodilation presented here results from different mechanisms of coronary and peripheral vasodilation induced by this compound.

Noteworthy, lack of stereoselectivity of nebivolol to induce coronary vasodilation speaks against involvement of any receptors in this response. Intracellular endothelial mechanisms are more likely to be primarily involved. Indeed, nebivolol altered inositol phosphate pathways (3) and increased intracellular calcium level in endothelium (14).

Peripheral endothelial action of nebivolol was proposed to be mediated by endothelial β2-, β3-adrenoceptors or 5 HT1A receptors (9-11). Interestingly, other β-adrenoceptor antagonists were shown to interact with 5-HT receptors (16). Here we directly exclude any role of these receptors in the mechanism of coronary vasodilation induced by nebivolol. This statement is based on the fact that the nonselective β1/β2-adrenoceptor antagonist - nadolol (10⁻⁵ M), the selective β3-adrenoceptor antagonist - L 748337 (10⁻⁶ M) (17) and the 5 HT1A receptor antagonist - NAN 190 (5 x 10⁻⁶ M) (11), none of them inhibited coronary vasodilation induced by D- and L-nebivolol. Moreover, another selective 5 HT1A

![Fig. 3. Lack of effect of nadolol (10⁻⁵ M) on coronary flow responses to D- nebivolol and L-nebivolol (3 x 10⁻⁵ M). Data are expressed as an increase in coronary flow (ml min⁻¹). Results are expressed as mean±SEM (n = 3).](image-url)
receptor antagonist WAY 10063 (10^6 M) (18) was also without an effect on vasodilation induced by D- or L-nebivolol (data not shown).

The lack of effects of these receptor antagonists on nebivolol-induced response cannot be explained by using insufficient concentrations. In control experiments, L-748337 (10^6 M) diminished vasorelaxation induced by β_1-adrenoceptor agonist (BRL 37344) in guinea pig aorta (data not shown). In isolated guinea pig heart nadolol (10^5 M) abolished an increase in dP/dt induced by isoprenaline (1 nmole). Also in this preparation vasodilator responses to serotonin were reduced by NAN 190 (5 x 10^6 M) (Fig. 5) or by WAY 10063 (10^-6 M) (data not shown). Importantly, we used higher concentration of NAN 190 as compared to Kakoki et al (11) who showed that NAN 190 at a concentration of 10^6 M would inhibit vasodilator response of nebivolol in the isolated rat kidney. This was due to the fact that in our hands 10^6 M of NAN 190 failed to inhibit significantly coronary vasodilation induced by serotonin. Lack of involvement of serotonin receptors in nebivolol response may be also supported by the fact that kinetics of coronary flow increase in response to serotonin and nebivolol differed substantially (Fig. 5). Taking these findings together we conclude that nebivolol-induced coronary vasodilation is not mediated by β_2, β_3 adrenoceptor or 5HT_1A receptors.

It was reported previously, that in murine aorta nebivolol (or its metabolite) induced NO-dependent relaxation via stimulation of endothelial β_2-adrenoceptors

![Graph](attachment:image.png)

Fig. 4. Lack of effect of 748337 (10^6 M) on coronary flow responses to D-nebivolol and L-nebivolol (3 x 10^-5 M). Data are expressed as an increase in coronary flow (ml min^-1). Results are expressed as mean±SEM (n = 3).
(9). In contrast with aorta or peripheral circulation, where stimulation of endothelial β₂-adrenoceptor induces vasodilation (8), this mechanisms is unlikely to be of importance in the coronary circulation. In coronary arteries β₁-adrenoceptors, but not β₂-adrenoceptors dominate (19). So, it was not surprising for us to find that coronary vasodilation induced by each stereoisomer of nebivolol was not inhibited by β₁/β₂-adrenoceptor antagonist - nadolol.

In cultured endothelial cells nebivolol-induced rise in c-AMP was inhibited by a β₁/β₂/β₃ adrenoceptor antagonist bupranolol and by a selective β₁-adrenoceptor antagonist - S(-)-cyanopindolol (10). Recently, we have shown that β₁-
adrenoceptor did not play any role in the regulation of coronary flow in the isolated guinea pig heart (Kozlovski et al., in press). Therefore, the lack of involvement of \( \beta_3 \)-adrenoceptor receptor in nebivolol induced response in isolated guinea pig heart is in line with our previous findings.

In conclusion, D-nebivolol and L-nebivolol are similar in their potency to induce nitric oxide-mediated coronary vasodilation in the isolated guinea pig heart. Neither \( \beta \)-adrenoceptors nor 5 HT\(_{1A} \) receptors are involved in this response. We suppose that nebivolol-induced simulation of coronary endothelium is not a receptor-dependent response but rather it involves a direct stimulation of intracellular endothelial mechanisms. Whether pleiotropic action of nebivolol in coronary endothelium is a pharmacological entity distinct from its peripheral vasodilator properties is a question waiting to be answered.

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**REFERENCES**


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