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Effect of Nebivolol on Isolated Rat Bladder Strips Precontracted with Carbachol or Potassium Chloride

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ABSTRACT

Objective: One of the proposed mechanism mediating the vasorelaxant effect of nebivolol is based on its agonistic activity on beta-2 and/or beta-3 adrenergic receptors. These receptors are also involved in the relaxation of urinary bladder. The aim of this study was to explore the ability of nebivolol to induce relaxation of the isolated rat bladder strip precontracted with cholinergic stimuli using carbachol or non-cholinergic stimuli using potassium chloride (KCl).

Methods: The isolated bladder strips were mounted in organ bath and contracted by KCl (40 μM) or carbachol (1mM) before the cumulative addition of nebivolol (0.0001-100 μM). To investigate the role of beta-adrenergic receptors in the nebivolol-induced relaxant response, some bladder strips were incubated with propranolol (1 μM) for 30 min. Statistical significance was tested by Student's *t*-test. $p<0.05$ was considered to be statistically significant.

Results: Nebivolol elicited concentration-dependent relaxant response in the bladder strips precontracted with KCl or carbachol. Although the relaxant response to nebivolol in the bladder strips precontracted with carbachol was significantly inhibited by propranolol ($p<0.05$), the nebivolol-induced relaxation failed to be inhibited by propranolol in the bladder strips precontracted with KCl. The maximum relaxation in response to nebivolol was found to be significantly higher in the bladder strips precontracted with carbachol compared to that of KCl ($p<0.05$).

Conclusion: The findings of the present study indicate that beta-adrenergic receptors play role in the relaxant response of nebivolol in the isolated rat bladder strip precontracted with carbachol.

Keywords: Nebivolol, relaxation, carbachol, potassium chloride, bladder, rat

1. INTRODUCTION

Nebivolol is a selective beta-1 adrenergic receptor (β_1-AR) blocker differently from conventional β-blockers because of its vasodilator and antioxidant properties (1,2). Although the precise mechanisms by which nebivolol induces relaxation are not completely understood, there is increasing evidence showing that nitric oxide (NO) production by endothelium-dependent mechanisms including β_2 – and/or β_3-ARs are thought to be primarily responsible for the vasorelaxant effect of nebivolol (3,4).

There is substantial evidence to indicate that β-ARs also involve in the relaxation of urinary bladder (5-7). Further evidence may come from the pharmacological studies which showed that the relaxant response to isoproterenol and other non-selective β-agonists is associated with β_2 – and/or β_3-ARs in the rat bladder (8, 9).

The physiological voiding function of urinary bladder is controlled by the contraction of urinary bladder smooth muscle (10). This contraction is primarily elicited by muscarinic receptor stimulation by acetylcholine released from parasympathetic nerve endings (10,11). However, under pathophysiological conditions, both cholinergic and non-cholinergic stimulus play role in the bladder contraction (12).

The present study was designed to investigate the effect of nebivolol on the isolated rat urinary bladder precontracted with either cholinergic or non-cholinergic stimulus induced by carbachol or potassium chloride (KCl), respectively.

2. METHODS

2.1. Drugs and Chemicals

Nebivolol hydrochloride, carbachol and propranolol hydrochloride were obtained from Sigma-Aldrich (USA). All compounds were dissolved in distilled water except nebivolol. Nebivolol was dissolved in dimethylsulphoxide and the final concentration of the solvent in the organ bath was less than 0.01% (v/v).

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2.2. Experimental Animals

Experimental protocols were approved by KOBAY DHL Inc. Ethical Committee for Experimental Research on Animals (Approval date and number: 14.01.2020/451). Male Sprague–Dawley rats (250-300g, n=6) were used in this study. The rats were housed in cages and were allowed ad libitum access to standard laboratory diet and tap water.

2.3. Experimental Design

The anesthetized rats were sacrificed by cervical dislocation. Thereafter, the urinary bladder was rapidly excised and placed in Krebs–Henseleit solution (composition in mM: NaCl, 118; KCl, 4.7; MgSO4•7H2O, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; and glucose, 11). The bladder was cleaned from surrounding adjacent adipose and soft connective tissue. Afterwards, the upper most dome and the lower trigone area were removed and the remaining body of the bladder was sliced longitudinally into approximately 2 × 10 mm strips. The isolated bladder strips were placed in organ bath chambers attached to force displacement that were connected to a computer for isometric force recording. The Krebs–Henseleit solution in the bath was continuously aerated by mixture of 95% O_2 and 5% CO_2, and maintained at 37°C. Resting tension of strips was set to 1 g and allowed to equilibrate for 60 minutes with replacing fresh Krebs–Henseleit solution every 15 min. To investigate whether the relaxant effect of nebivolol may differ in bladder strips due to distinct precontractile stimulus, carbachol for muscarinic receptor activation or KCl for membrane depolarization was used for this purpose. After the equilibration period, nebivolol (0.0001–100 µM) was cumulatively added to organ bath to obtain cumulative concentration response curves (CCRCs) of the bladder strips precontracted with KCl (40 mM, n=6) or carbachol (1 µM, n=5) and served as controls. To test the role of β-ARs in this response, some bladder strips were incubated for 30 min with propranolol (non-selective β-AR antagonist, 1 µM).

2.4. Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). Relaxation is expressed as the percentage of the contraction caused by KCl or carbachol. Efficacy of nebivolol was expressed as maximum relaxation (E_{max}). The analysis was performed using the statistical software package (GraphPad Prism, USA). Statistical significance was tested by Student's t-test. Differences were considered to be statistically significant when $p<0.05$.

3. RESULTS

Nebivolol elicited a concentration dependent relaxation in the KCl-precontracted bladder strips (n=6). However, there is no significant inhibition in the nebivolol-induced relaxation by the presence of propranolol (Figure 1, $p>0.05$).

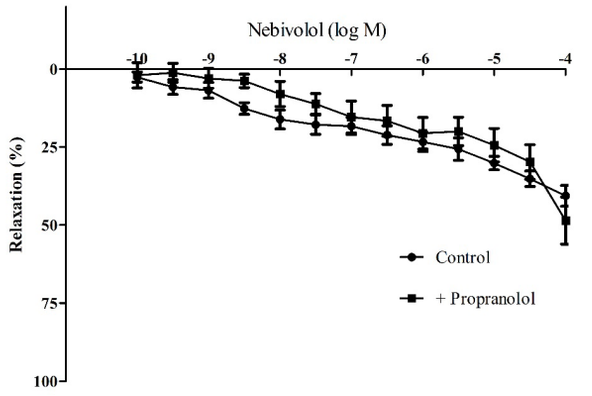

Figure 1. *Nebivolol-induced relaxation in the rat bladder strips precontracted with KCl (40 mM) in the absence (Control, n=6) or presence of propranolol (+Propranolol, 1 mM, n=5)*

Nebivolol also caused relaxation in the carbachol-precontracted bladder strips in a concentration-dependent manner (n=5). Additionally, the relaxant response to nebivolol was significantly inhibited by the presence of propranolol in the isolated bladder strips (Figure 2, $p<0.05$).

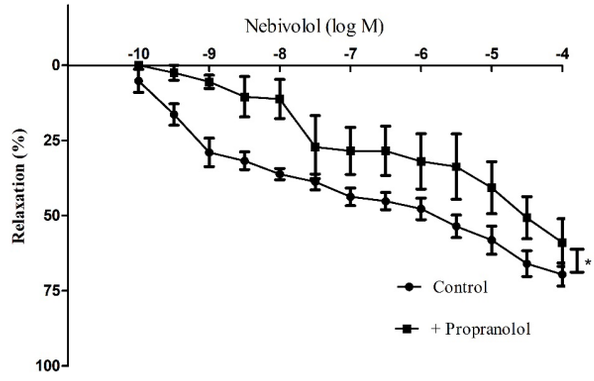

Figure 2. *Nebivolol-induced relaxation in the rat bladder strips precontracted with carbachol (1 µM) in the absence (Control, n=5) or presence of propranolol (+Propranolol, 1 mM, n=4). p<0.05 vs control*

The maximum relaxation (E_{max}) in response to nebivolol was significantly higher when bladder strips were precontracted with carbachol than that of KCl (Figure 3, $p<0.05$).

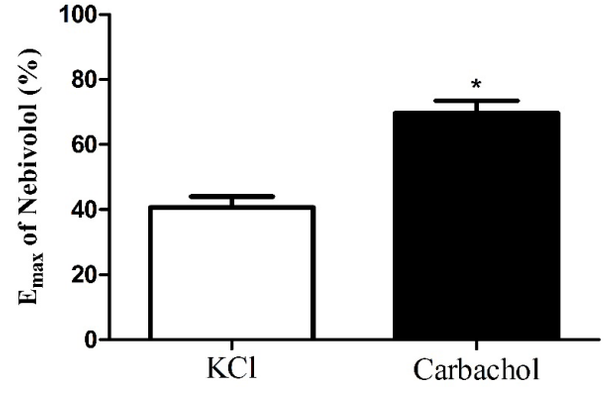

Figure 3. *E_{max} of nebivolol in the rat bladder strips precontracted with KCl (40 mM, n=6) or carbachol (1 µM, n=5). p<0.05 vs KCl*

4. DISCUSSION

In the present study, the relaxation induced by nebivolol was concentration-dependent both in the KCl-precontracted or carbachol-precontracted isolated bladder strips. However, this response to nebivolol was found to be more efficacious when the precontracted stimulus was carbachol instead of KCl. Additionally, the concentration-dependent relaxant effect of nebivolol was inhibited by propranolol in the bladder strips precontracted with carbachol.

As mentioned before, the contractions in the bladder evoked by carbachol or KCl indicate cholinergic and non-cholinergic stimulus, respectively. The present data showing a higher efficacy of nebivolol in the bladder strips precontracted with carbachol indicate that distinct mechanisms involve in the strength of precontraction contribute to differences in the ability of nebivolol to induce relaxation. The mechanisms responsible for the differences will be investigated in future studies, but one possible explanation of these findings is that different calcium sources play role in the contractile response to various stimulus in the bladder smooth muscle (13). Although the KCl-induced contraction mainly depends on entry of calcium from extracellular sources, both calcium from extracellular sources and release from sarcoplasmic reticulum mediate the contractile response to carbachol in the bladder smooth muscle (13). Nebivolol has been reported to possess an inhibitor action on the both calcium sources and this may partly explain the question why nebivolol is more efficacious in the bladder strips precontracted with carbachol (14,15).

The β-ARs are thought to be involved in the relaxation of the urinary bladder smooth muscle (16,17). For this reason, some bladder strips were incubated with propranolol before addition of nebivolol cumulatively to the organ bath in the present study. Interestingly, propranolol inhibited the relaxant response to nebivolol in the bladder strips precontracted with carbachol but failed to inhibit this response in the KCl-precontracted bladder strips. The present data support the hypothesis that β-ARs are involved in the nebivolol-mediated relaxation in the isolated urinary bladder strips precontracted with a cholinergic stimuli. However, the contribution of each subtype (especially β_2 – and β_3-ARs) in this action is needed to be elucidated by further studies.

The present data are in line with a previous study showing nebivolol-induced relaxation in the rat bladder strips precontracted with KCl (18). In the same study, it has also been shown that this response to nebivolol was insensitive to SR 59230A (β_3-AR antagonist) (18). However, to the best of knowledge, there is no study evaluating the effects of nebivolol on the bladder strips precontracted with carbachol.

As previously mentioned, NO has been shown to be involved in the nebivolol-mediated vasorelaxation through endothelial β_2 – and/or β_3-ARs (3, 4). Additionally, NO has also been reported to play a role in the relaxation of isolated trigonal and urethral preparations (19). However, in the human detrusor smooth muscle, it has been found that the relaxation induced by β-AR agonist might not relate with NO release (20). Although not addressed in the present study, the contribution of NO in the bladder relaxation through nebivolol is still question.

In conclusion, the present results show that nebivolol could produce more efficacious relaxation in the rat bladder strips precontracted with carbachol than that of KCl. Additionally, these findings suggest that relaxation elicited by nebivolol is involved in the β-ARs in the isolated rat urinary bladder strips precontracted with a cholinergic stimuli.

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