

## PAPER DETAILS

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## RESEARCH ARTICLE

# The Involvement of ATP-Sensitive Potassium Channels in the Nebivolol-Induced Relaxation of Endothelium-Intact Aorta Isolated from Rats

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## Abstract

**Objective:** Nebivolol is a highly selective beta-1 adrenergic receptor blocker with additional vasorelaxant properties. The vasorelaxant effect of nebivolol has been mainly attributed to endothelium-dependent mechanisms including beta-adrenergic receptors. However, the involvement of ATP-sensitive potassium ( $K_{ATP}$ ) channels, another potential mechanism for vasorelaxant effect, in the vasorelaxant response to nebivolol remains unclear. Therefore, this study was aimed to investigate the role of  $K_{ATP}$  channels in the nebivolol-induced vasorelaxation in the isolated rat aorta

**Methods:** The rat thoracic aortic rings isolated from Sprague-Dawley rats were mounted in organ bath chambers containing Krebs-Henseleit solution at 37 °C continuously bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. After an equilibration period, the presence of endothelium was confirmed by the response (more than 50%) to acetylcholine (10 µM) in aortic rings precontracted with phenylephrine (1 µM). After washout, in control group, the endothelium-intact aortic rings were contracted by potassium chloride (30 mM) before the cumulative addition of nebivolol (0.0001-100 µM). In some experiments, the relaxant response to nebivolol (0.0001-100 µM) was also obtained in the presence of glibenclamide ( $K_{ATP}$  channel blocker, 10 µM) or N<sup>ω</sup>-Nitro-L-arginine methyl ester (L-NAME: eNOS inhibitor, 100 µM) in the endothelium-intact aortic rings precontracted with potassium chloride (30 mM). Data were presented as means±SEM. Multiple comparisons of groups were performed by using ANOVA followed by post-hoc Bonferroni test.

**Results:** Nebivolol elicited a concentration dependent vasorelaxant effect in the endothelium-intact aortic rings. Relaxant response to nebivolol was significantly inhibited by the presence of glibenclamide or L-NAME ( $p < 0.05$ ). Although  $E_{max}$  values were not found significantly different among groups,  $pD_2$  values of nebivolol were reduced in the endothelium-intact aortic rings incubated with glibenclamide or L-NAME.

**Conclusion:** These results demonstrate for the first time the involvement of  $K_{ATP}$  channels in the nebivolol-induced vasorelaxation in the endothelium-intact aorta precontracted with potassium chloride.

**Key words:** Nebivolol, vasorelaxation, ATP-sensitive potassium channels, nitric oxide, rat aorta.

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## Introduction

Nebivolol is a third-generation, cardioselective beta ( $\beta$ )-blocker with additional antioxidant and vasorelaxant properties. The latter effect of nebivolol is closely associated with nitric oxide (NO) production by endothelium-dependent mechanisms (Cicero et al., 2018; Olawi et al., 2019).

The involvement of the endothelium in the vascular tone has been widely documented (Félétou M and Vanhoutte, 2006). In this regard, there is increasing evidence showing that endothelial beta-2 ( $\beta_2$ )- and/or  $\beta_3$ -ARs are primarily responsible for the vasorelaxant response to nebivolol (Broeders et al., 2000; de Groot et al., 2003). These receptors belong to the G-protein coupled receptor family (Biaggioni and Robertson, 2018).

Potassium channels including ATP-sensitive potassium ( $K_{ATP}$ ) channels play an important role in the vascular tonus due to their effects on membrane potential. The opening of  $K_{ATP}$  channels results in membrane hyperpolarization in smooth muscle and hence contributes to vasorelaxation (Sobey, 2001). Recently,  $K_{ATP}$  channels are reported to be also present in the vascular endothelium and have a functional role in the vascular reactivity (Aziz et al., 2017). The vasodilatory action of adenosine through its receptors has also been shown to be associated with activation of endothelial  $K_{ATP}$  channels (Kuo and Chancellor, 1995). Adenosine receptors are also coupled with stimulatory G-protein like  $\beta_2$ - and  $\beta_3$ -ARs (Biaggioni and Robertson, 2018).

Most studies investigating vascular action of nebivolol have mainly focused on adrenergic receptors linking with NO production to explain the mechanism of vasorelaxation. Experimental evidence regarding the role  $K_{ATP}$  channels in the vasorelaxant effect of nebivolol is still limited. Therefore, it would be informative to investigate the role of  $K_{ATP}$  channels in the vasorelaxant effect of nebivolol. This study was aimed to investigate whether  $K_{ATP}$  channels could involve in the vasorelaxant response to nebivolol in the endothelium-intact aorta isolated from rat.

## Methods

### Drugs and chemicals

Nebivolol hydrochloride, phenylephrine hydrochloride (PE), acetylcholine hydrochloride (ACh) and N $\omega$ -Nitro-L-arginine methyl ester hydrochloride (L-NAME) were obtained from Sigma-Aldrich. In addition, glibenclamide was obtained from Tocris. Nebivolol and glibenclamide was dissolved in dimethylsulphoxide as described previously (Rautureau et al., 2002; Pullen et al., 2014)

and the final concentration of the solvent in the organ bath was less than 0.01% (v/v).

### Animals

Experimental protocols were approved by Ethical Committee for Experimental Research on Animals. Male Sprague–Dawley rats (250–300g) were used in this study. The rats were housed in cages under 12/12 hours light/dark cycle and were allowed ad libitum access to standard laboratory diet and tap water.

### Preparation of Rat Thoracic Aortic Rings

The anesthetized rats were sacrificed by cervical dislocation and the descending thoracic aorta was rapidly dissected out and placed in Krebs-Henseleit solution (KHS) composed of (mM): NaCl, 118; KCl, 4.7; MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.2; KH<sub>2</sub>PO<sub>4</sub>, 1.2; CaCl<sub>2</sub>, 2.5; NaHCO<sub>3</sub>, 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<sub>2</sub> +5% CO<sub>2</sub>) and attached to force displacement that were connected to a computer for isometric force recording.

### Experimental protocol

The aortic ring was held at a resting tension of 2 g and allowed to equilibrate for 1 h with washing fresh KHS every 15 min. After this equilibration period, the integrity of the vascular endothelium was checked by contracting the tissues with PE (1  $\mu$ M) and adding ACh (10  $\mu$ M). Only tissues that relaxed by more than 50% to ACh were included in this study.

In order to elucidate the impact of  $K_{ATP}$  channels in the nebivolol-mediated vasorelaxation, some aortic rings were incubated with the glibenclamide ( $K_{ATP}$  channel blocker, 10  $\mu$ M, n=6) for 30 minutes. Additionally, the NO-dependent effect of nebivolol was also evaluated by the incubation of some aortic rings with L-NAME (endothelial nitric oxide synthase inhibitor, 100  $\mu$ M, n=4) for 20 minutes. Other aortic rings were not incubated and served as controls (n=5). Nebivolol (10-10-10-4 M) was cumulatively added to organ bath to obtain cumulative concentration-relaxation curves (CCRCs) in the aortic rings precontracted with potassium chloride (KCl, 30 mM). The concentrations of antagonists were selected based on preliminary experiments and previous studies (Sobey, 2001; Aziz et al., 2017).

## Statistical analysis

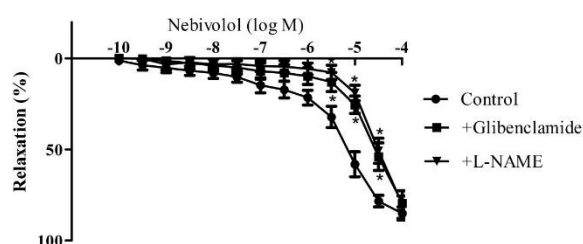
Data are expressed as mean  $\pm$  SEM. Relaxation is expressed as the percentage of the contraction caused by KCl. The analysis was performed using the statistical software package (Graphpad Prism, USA). Statistical significance was tested by ANOVA followed by the Bonferroni Comparison post-test. Efficacy of nebivolol was expressed as maximum relaxation ( $E_{max}$ ) and determined as a percentage of the KCl precontraction.  $pD_2$  (negative logarithm of the half maximum effective concentration ( $EC_{50}$ )) values were calculated for potency of nebivolol. Differences were considered to be statistically significant when  $p < 0.05$ .

## Results

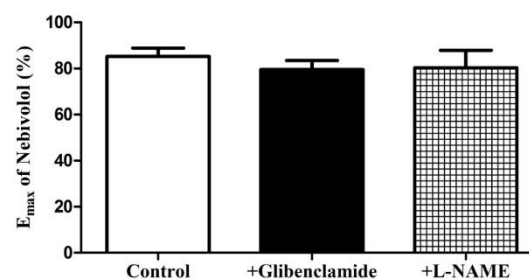
Nebivolol produced concentration-dependent relaxation in the rat aorta precontracted with KCl (Fig 1,  $n=5$ ). The vasorelaxant effect of nebivolol was significantly inhibited after incubation of aortic rings with glibenclamide (10  $\mu$ M,  $n=6$ ) or L-NAME (100  $\mu$ M,  $n=4$ ). (Fig 1,  $*p < 0.05$ ). Additionally, as shown in Figure 1, incubation of aortic rings with glibenclamide (10  $\mu$ M) or L-NAME (100  $\mu$ M) caused a rightward shift of the CCRCs.

The  $E_{max}$  values for nebivolol were not significantly different in the aortic rings incubated with glibenclamide (10  $\mu$ M,  $n=6$ ) or L-NAME (100  $\mu$ M,  $n=4$ ) compared to controls ( $n=5$ ) (Fig 2,  $p > 0.05$ ).

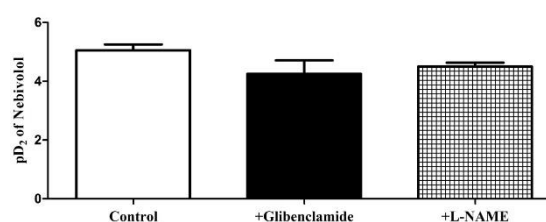
The potency of nebivolol as attested by  $pD_2$  values were reduced by the presence of glibenclamide (10  $\mu$ M,  $n=6$ ) or L-NAME (100  $\mu$ M,  $n=4$ ) (Fig 3).



**Figure 1.** Percentage relaxations of endothelium-intact aortic rings in response to cumulative concentrations of nebivolol in the absence ( $n=5$ ) or presence of glibenclamide (10  $\mu$ M,  $n=6$ ) or L-NAME (100  $\mu$ M,  $n=4$ ).  $*p < 0.05$  vs Control.



**Figure 2.**  $E_{max}$  of nebivolol in the the absence ( $n=5$ ) or presence of glibenclamide (10  $\mu$ M,  $n=6$ ) or L-NAME (100  $\mu$ M,  $n=4$ ) in the endothelium-intact aortas.



**Figure 3.**  $pD_2$  of nebivolol in the the absence ( $n=5$ ) or presence of glibenclamide (10  $\mu$ M,  $n=6$ ) or L-NAME (100  $\mu$ M,  $n=4$ ) in the endothelium-intact aortas.

## Discussion

In the present study, nebivolol produced NO-dependent vasorelaxation in the endothelium-intact aorta precontracted with KCl. In addition, the present findings provide a new mechanism showing the involvement of  $K_{ATP}$  channels in the vasorelaxant response to nebivolol in the endothelium-intact aorta.

$K_{ATP}$  channels play an important regulator role in the vascular tonus by their ability to couple cellular metabolism with membrane potential (Aziz et al., 2017). In this regard, membrane hyperpolarization induced by activation of sarcolemmal vascular smooth muscle  $K_{ATP}$  channels (sarc $K_{ATP}$  channels) results in the vasorelaxant response (Sobey, 2001). Therefore, most in vitro experiments were focused to clarify that the role of sarc $K_{ATP}$  channels in the vasorelaxant response using the endothelium-denuded aortic preparations (Pantan et al., 2014; Arsyad and Dobson, 2016). However, endothelial  $K_{ATP}$  channels also exist (Aziz et al., 2017) and the vasorelaxant effect may be evaluated in the presence of both endothelial  $K_{ATP}$  and sarc $K_{ATP}$  channels to obtain whole vascular response. Although the functional role of sarc $K_{ATP}$  channels is well known, there is limited information regarding the involvement of endothelial  $K_{ATP}$  channels in vascular function. Indeed, it has been reported that  $K_{ATP}$  channels are also present in the vascular endothelium

and contribute to the vascular reactivity in the coronary arteries (Aziz et al., 2017). Structurally,  $K_{ATP}$  channels consist of subunits including pore-forming inward rectifier Kir6.x subunits (Kir6.1 or Kir6.2) and regulatory sulfonylurea receptors (SUR1, SUR2A, or SUR2B) (Tinker et al., 2014). The different combinations of these subunits form  $K_{ATP}$  channels which have distinct pharmacological and electrophysiological properties as in vascular and pancreatic beta cells (Aziz et al., 2015; Ashcroft et al., 2017). Aziz et al. found that Kir6.1 subtype is the relevant subunit in the endothelial  $K_{ATP}$  channels similar to sarc $K_{ATP}$  channels (Aziz et al., 2017). Moreover, endothelial  $K_{ATP}$  channels are also found to be partly involved in the vasodilatory action of adenosine (Kuo and Chancellor, 1995). This seems to be associated with direct phosphorylation of these channels via protein kinase A which is activated by stimulation of G-protein coupled adenosine receptors by adenosine (Aziz et al., 2017). The activation is also pronounced through distinct stimulatory G-protein coupled receptors such as  $\beta$ -adrenergic receptors ( $\beta$ -ARs) and this mechanism is involved in  $\beta$ -AR-mediated vasorelaxant response (Biaggioni and Robertson, 2018). Nebivolol produces an endothelium-dependent vasorelaxant effect primarily attributed to endothelial  $\beta_2$ - and/or  $\beta_3$ -ARs stimulation and subsequent activation of eNOS (Broeders et al., 2000; de Groot et al., 2003). NO is an endothelium-derived relaxing factor which increases the production of cyclic guanosine monophosphate (cGMP) by activating of soluble guanylate cyclase in the vascular smooth muscle (Vanhoutte et al., 2017). Alternatively, NO could also induce hyperpolarization in the smooth muscle of different arteries, including the rat aorta (Vanheel et al., 1994).

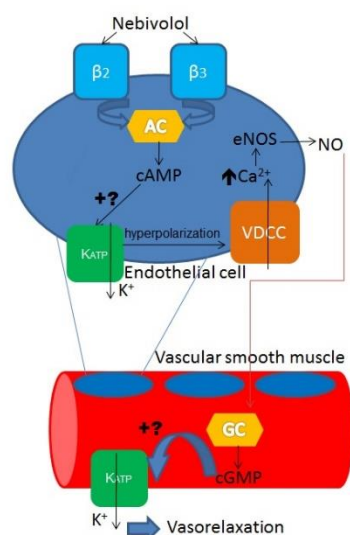
A critical question is whether  $K_{ATP}$  channels play any role in the nebivolol-induced relaxation due to their effects on membrane potential in the aorta precontracted by KCl-mediated membrane depolarization. The vasorelaxant activity of nebivolol seems to be primarily based on increased endothelial NO production by stimulation of endothelial  $\beta_2$ - and/or  $\beta_3$ -ARs, as mentioned before. However, the NO production induced by nebivolol may also be under the modulation of endothelial  $K_{ATP}$  channels. This hypothesis is supported by the findings of present study showing a similar inhibition of nebivolol-induced vasorelaxant response in the presence of  $K_{ATP}$  channel blocker or endothelial nitric oxide synthase inhibitor. This outcome is also supported by another study which shows that NO-induced changes in membrane potential were

inhibited by  $K_{ATP}$  channel blocker (Garland and McPherson, 1992). Unfortunately, a limitation of the present study was the lack of measurement of membrane potentials due to the insufficiency of technical resources at the laboratory.

In previous organ bath experiments, nebivolol has been similarly found to induce relaxation in the rat aorta precontracted with KCl (de Groot et al., 2003; Wang et al., 2009). Additionally, they have also shown that this effect is NO-dependent because it could be abrogated by endothelial NO synthase inhibitor (de Groot et al., 2003; Wang et al., 2009). However, Wang et al. (2009) found that glibenclamide failed to inhibit nebivolol-induced relaxation in the rat aorta. This discrepancy may be explained by concentrations of glibenclamide and KCl that differ from the present study. Because, the appropriate concentrations of glibenclamide and KCl were selected based on a preliminary experiments of the present study. For example, the concentration of KCl (30 mM) was tested and selected because it has been found that nebivolol was failed to induce relaxation when higher concentration (60 mM) of KCl was used in the experimental protocol (data not shown). In addition to this, the concentrations of glibenclamide and KCl used in the present study were in line with previous in vitro studies in the rat aorta (Ito et al., 1997; Erdei et al., 2006; Mateus et al., 2019).

### Conclusion

In conclusion, the present findings suggest for the first time that activation of  $K_{ATP}$  channels is involved in the relaxant response to nebivolol in the rat aorta precontracted with KCl. The vasorelaxant effect of nebivolol is also found to be NO-dependent. Taken together, possible mechanisms by which NO and  $K_{ATP}$  channels could involve in the nebivol-induced relaxation in the endothelium-intact aortas were summarized in the Fig 4. However, further studies are needed to clarify the signalling pathways including endothelial and sarcolemmal  $K_{ATP}$  channels in the nebivolol-induced vasorelaxation.



**Fig 4.** Possible mechanisms leading to nebivolol-induced relaxation in the endothelium-intact aorta.

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**Author Contributions:** Concept HOAC; Design HOAC; Supervision HOAC; Materials HOAC; Data collection and/or Processing HOAC; Analysis and/or Interpretation HOAC; Literature Review HOAC; Writing HOAC; Critical Review HOAC.

**Conflict of Interest:** No conflict of interest was declared by the author.

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