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RESEARCH ARTICLE

Potassium Channels as Resilient Targets for Airway Smooth Muscle Relaxation Under Inflammatory Stress

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SUMMARY

Airway inflammation may reduce β_2-adrenoceptor-mediated bronchodilation, underscoring the need to investigate receptor-independent mechanisms of airway smooth muscle (ASM) relaxation in asthma. Pharmacological activation of potassium channels can promote membrane hyperpolarization and limit voltage-dependent Ca^{2+} influx, providing a potential alternative pathway for bronchodilation. This study compared the relaxation responses to isoprenaline and four pharmacological potassium channel activators in a murine model of LPS-induced airway inflammation. Mice were administered phosphate-buffered saline (PBS) or LPS, and the isolated tracheal rings were subsequently precontracted with carbachol. Cumulative concentration–response curves were generated for isoprenaline, NS1619 (BK_{Ca} activator), NS309 (K_{Ca2}/$K_{Ca3.1}$ activators), flupirtine (K_v7 activator), and pinacidil (K_{ATP} activator). In tracheal rings from PBS-treated mice, all four potassium channel activators elicited concentration-dependent relaxation with comparable response profiles. The principal finding was that LPS treatment significantly attenuated isoprenaline-induced relaxation, whereas the responses to NS1619, NS309, flupirtine, and pinacidil were not significantly altered. This differential effect suggests that the pharmacological pathways recruited by direct potassium channel activation remain functionally responsive in this LPS model, despite impaired β_2-adrenoceptor-mediated relaxation. However, because the channel activity, membrane potential, and β_2-adrenoceptor signaling components were not measured directly, the underlying mechanism and site of impairment could not be determined from the present functional data. These findings identify potassium channels as potential alternative or adjunctive bronchodilator targets in inflammatory airway diseases, particularly under conditions of reduced β_2-agonist responsiveness. Further studies using more selective pharmacological tools, electrophysiological measurements, and human airway preparations are required to establish the target specificity and translational relevance.

Keywords: *Airway smooth muscle, potassium-channel openers, isoprenaline, lipopolysaccharide, bronchodilation.*

İnflamatuvar Stres Altında Hava Yolu Düz Kasının Gevşemesinde İşlevini Koruyan Hedefler Olarak Potasyum Kanalları

ÖZ

Solunum yolu inflamasyonu, β_2-adrenoseptör aracılı bronkodilatasyonu azaltabileceğinden, solunum yolu düz kasını reseptörden bağımsız mekanizmalarla gevşeten alternatif yaklaşımlara gereksinim duyulmaktadır. Potasyum kanallarının farmakolojik aktivasyonu, membran hiperpolarizasyonunu artırarak voltaja bağımlı Ca^{2+} girişini sınırlayabilir. Bu çalışmada, lipopolisakkarit (LPS) ile oluşturulan solunum yolu inflamasyonu modelinde izoprenalin ile farklı potasyum kanalı aktivatörlerinin gevşetici etkileri karşılaştırılmıştır. Dişi CD1 farelere intranazal olarak fosfat tamponlu salin veya LPS uygulanmış; 48 saat sonra izole edilen trakea halkaları karbakol ile önceden kastırılmıştır. İzoprenalin ile BK_{Ca} aktivatörü NS1619, K_{Ca2}/$K_{Ca3.1}$ aktivatörü NS309, K_v7 aktivatörü flupirtin ve KATP aktivatörü pinasidilin kümülatif konsantrasyon–yanıt eğrileri elde edilmiştir. Kontrol grubunda dört potasyum kanalı aktivatörü de konsantrasyona bağlı ve birbiriyle karşılaştırılabilir gevşeme yanıtı oluşturmuştur. LPS uygulaması, izoprenalinin konsantrasyon–yanıt eğrisini anlamlı biçimde sağa kaydırmış ancak maksimum gevşeme yanıtını değiştirmemiştir. Buna karşılık NS1619, NS309, flupirtin ve pinasidil ile oluşturulan gevşeme yanıtları LPS uygulamasından anlamlı düzeyde etkilenmemiştir. Bu bulgular, β_2-adrenoseptör aracılı gevşeme duyarlılığının azaldığı inflamatuvar koşullarda, doğrudan potasyum kanalı aktivasyonuyla uyarılan gevşetici mekanizmaların işlevsel olarak korunduğunu göstermektedir. Potasyum kanalları, β_2-agonistlere yanıtın azaldığı inflamatuvar solunum yolu hastalıklarında reseptörden bağımsız veya tamamlayıcı bronkodilatör hedefler sunabilir. Bununla birlikte, hedef özgüllüğünün ve klinik uygulanabilirliğin belirlenmesi için daha seçici bileşikler ve insan solunum yolu dokuları kullanılarak ileri çalışmalar yapılmalıdır.

Anahtar Kelimeler: *Solunum yolu düz kası, potasyum kanalı aktivatörleri, izoprenalin, lipopolisakkarit, bronkodilatasyon*

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INTRODUCTION

Airway smooth muscle (ASM) is a major determinant of airway tone and responsiveness, integrating the effects of endogenous bronchoconstrictor and bronchodilator mediators, as well as therapeutic agents (Xiong, Martin, & Lauzon, 2022). Epithelium-derived prostanoids and gasotransmitters, including nitric oxide and hydrogen sulfide, contribute to this regulation by modulating ASM membrane potential and intracellular Ca^{2+} signaling (Matsumoto et al., 1994). ASM contraction depends on increases in cytosolic Ca^{2+} resulting from the release from intracellular stores and influx across the plasma membrane, whereas relaxation is facilitated by membrane hyperpolarization, which limits voltage-dependent Ca^{2+} entry and reduces Ca^{2+} availability (Jude, Wylam, Walseth, & Kannan, 2008; Khalfaoui & Pabelick, 2023).

Four principal potassium-channel groups have been implicated in ASM hyperpolarization and relaxation: large-conductance Ca^{2+}-activated potassium (BK_{Ca}) channels; small- and intermediate-conductance Ca^{2+}-activated potassium channels (SK and IK_{Ca}; K_{Ca2} and $K_{Ca3.1}$, respectively); voltage-gated potassium (K_v7) channels; and ATP-sensitive potassium (K_{ATP}) channels (Orfali et al., 2023). These channels are also expressed in airway epithelial cells, fibroblasts, mast cells, and lymphocytes, suggesting broader roles in airway inflammation and remodeling (Liu & Xu, 2005). BK_{Ca} channels couple local Ca^{2+} signals to outward K^+ currents, although their contribution to intact human airway tone remains incompletely defined (Orfali et al., 2023). K_{Ca2} and $K_{Ca3.1}$ channels are voltage-independent and calmodulin-gated; $K_{Ca3.1}$ is primarily associated with ASM proliferation and remodeling, whereas the acute relaxant role of this channel group is less established (Bradding & Wulff, 2009). In contrast, K_{v7}.1, K_v7.4, and K_v7.5 generate a resting, M-current-like conductance that is suppressed by contractile agonists, including methacholine and histamine, and restored by pharmacological K_v7 activation (Brueggemann et al., 2012; Mondéjar-Parreño, Pérez-Vizcaíno, & Cogolludo, 2020). K_{ATP} channels represent one of the best-characterized potassium-channel pathways mediating ASM relaxation. These channels comprise Kir6.x pore-forming and sulfonylurea receptor regulatory subunits and couple cellular metabolic status to membrane excitability (Barreira et al., 2026). Collectively, these potassium channels provide mechanistically distinct targets for inducing ASM relaxation independently of conventional receptor-mediated bronchodilation (Liu & Xu, 2005; Orfali et al., 2023).

Isoprenaline and other β_2-adrenoceptor agonists induce ASM relaxation predominantly through the canonical Gαs–adenylyl cyclase–cyclic adenosine monophosphate (cAMP)–protein kinase A (PKA) pathway, which remains central to bronchodilator therapy in asthma (Shah et al., 2026). β_2-Adrenoceptor activation increases intracellular cAMP levels, leading to PKA-dependent modulation of contractile proteins, Ca^{2+} handling, and ion channel activity, thereby reducing intracellular Ca^{2+} availability and myofilament Ca^{2+} sensitivity (Barnes, 2011; Deshpande, Theriot, Penn, & Walker, 2008). However, airway inflammation may impair β_2-adrenoceptor-mediated airway smooth muscle relaxation. Experimental studies using isolated tracheal preparations have demonstrated that inflammatory cytokines can markedly attenuate β-agonist responses, whereas bacterial lipopolysaccharide (LPS) and viral stimuli may further compromise bronchodilator efficacy through the production of inflammatory mediators and disruption of β_2-adrenoceptor signaling (Guo et al., 2005; Rahman, Fitzpatrick, Oliver, & Bourke, 2014).

In the present study, we hypothesized that the relaxation induced by direct pharmacological activation of potassium channels would remain relatively preserved in LPS-exposed airways despite the attenuation of β_2-adrenoceptor-mediated relaxation. Accordingly, the present study compared the relaxant responses to isoprenaline and pharmacological activators of BK_{Ca},

$K_{Ca2}/K_{Ca3.1}$, K_v7, and K_{ATP} channels in isolated tracheal preparations in carbachol-precontracted tracheal rings isolated from control and LPS-treated mice.

MATERIALS AND METHODS

Experimental animals

A total of 59 female CD1 mice aged 10–12 weeks were used: 29 received phosphate-buffered saline (PBS) and 30 received lipopolysaccharide (LPS) in the present study. All procedures in the present study were approved by the Hacettepe University Animal Research Ethics Committee (No:2014-61/01).

Experimental airway inflammation protocol and drug treatment

LPS (*Escherichia coli* 0127:B8, Sigma-Aldrich, St. Louis, MO, USA) was applied to mice intranasally (i.n.) in order to induce airway inflammation (Starkhammar et al., 2012; 2014). For this purpose, 60 µl LPS (0.1 mg/mL in sterile PBS) to the LPS group and 60 µl PBS to the control group were applied to mice. 48 hours after LPS or PBS application, mice were sacrificed by cervical dislocation, and their tracheas were isolated.

Organ bath experiments

Tracheas were isolated from mice, the connective tissues were removed, and tracheal rings were prepared. The tracheal ring preparations were mounted in organ baths containing Krebs–Henseleit solution at 37 °C and gassed with 95% O_2-5% CO_2. The change in tension was measured with an isometric force transducer using the MP150-Biopac data acquisition system and AcqKnowledge 4.2 The tissues were equilibrated for 1 hour under a resting tension of 1 g by washing every 15 minutes. Contraction and relaxation responses were obtained in the presence of indomethacin (3 x 10^{-6} M), which was added to Krebs–Henseleit solution throughout the experimental protocol.

Evaluation of relaxation responses induced by isoprenaline and potassium channel openers

Carbachol (10^{-8}-10^{-4} M) contraction response was obtained from tracheal rings. Isoprenaline (10^{-9}-10_{-5}M), K_{ATP} opener pinacidil (10^{-8}-10^{-4} M), BK_{Ca} opener NS 1619 (10^{-7}-10^{-4} M), SK/IK_{Ca} opener NS309 (10^{-7}-10^{-4} M) and K_v7 opener flupirtine (10^{-7}-10^{-4} M) induced cumulative relaxation responses were evaluated in isolated tracheal rings which were pre-contracted with carbachol corresponding to 60-80% of maximal response. A single tracheal ring was isolated from each mouse and used to construct one concentration–response curve for a single pharmacological agent. Thus, each curve was generated using tissue from a different animal. Each experiment included six mice per treatment group, except for the pinacidil experiment, which included five PBS-treated and six LPS-treated mice.

Statistical analysis

Relaxation responses were represented as the percentage of pre-contractions. Maximal response (E_{max}) and pEC_{50} values were obtained from concentration-response curves. All data are presented as the mean ± standard error of the mean (SEM). Statistical analyses were performed using Student's t-test or two-way analysis of variance (ANOVA) followed by Tukey's multiple-comparisons test, as appropriate. $P < 0.05$ is considered significant. Data were analyzed using GraphPad Prism, version 5.01 (GraphPad Software Inc).

Materials

LPS (*Escherichia coli* O127:B8), isoprenaline (isoproterenol hydrochloride), pinacidil monohydrate, NS 1619 (1,3-Dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2H-benzimidazol-2-one), NS309 (3-Oxime-6,7-dichloro-1H-indole-2,3-dione), and flupirtine maleate were obtained from Sigma (St. Louis, MO, U.S.A).

RESULTS AND DISCUSSION

Relaxation response to isoprenaline was obtained in tracheal ring preparations isolated from LPS-treated and control groups of mice. LPS application leads to shifting of isoprenaline concentration-response curve to the right, and the pEC_{50} values were calculat-

ed as 7.54 ± 0.05 (n=6) for the LPS group and 7.84 ± 0.08 (n=6) for the control group, whereas maximum isoprenaline relaxation response was found similar in LPS and control groups. E_{max} values were calculated as 98.45 ± 1.57% for the LPS group and 106.59 ± 2.13% for the control group (Figure 1).

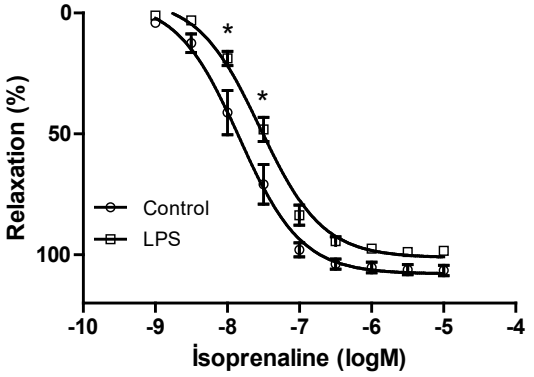

Figure 1. Effect of lipopolysaccharide (LPS) exposure on isoprenaline-induced relaxation in isolated mouse tracheal rings. Concentration-dependent relaxation responses to isoprenaline were obtained in carbachol-precontracted tracheal rings isolated 48 h after intranasal administration of phosphate-buffered saline (control) or LPS. Relaxation is expressed as a percentage of the carbachol-induced precontraction. Data are presented as the mean ± SEM (n=6 per group). Statistical comparisons were performed using two-way ANOVA followed by Tukey's multiple-comparisons test. *P<0.05 versus the corresponding control value

To explore the function of potassium channels, which are important in the regulation of airway tonus, we elicited the concentration-dependent relaxation responses induced with the BK_{Ca} opener NS 1619, SK/IK_{Ca} opener NS309, and K_v7 opener flupirtine in isolated tracheas obtained from LPS-and control groups of mice. The concentration-dependent relaxation responses induced with the BK_{Ca} opener NS 1619 (10^{-7}–10^{-4} M) were similar in LPS and control groups (Table 1 and Figure 2). Furthermore, there was no significant difference between the concentration-dependent relaxation responses induced with the SK/IK_{Ca} opener NS309 (10^{-7}–10^{-4} M) in tracheas isolated from LPS-and control groups (Table 1 and Figure 3). In addition, LPS application did not alter K_v7 opener flupirtine (10^{-7}–10^{-4}) induced relaxation responses (Table 1 and Figure 4). We assessed the function of potassium channels, which are important in the regulation of the resting membrane potential of the cell in accordance with metabolic processes. For this purpose, we elicited concentration-dependent relaxation responses induced with pinacidil (10^{-8}–10^{-4} M), and no differences were observed in pinacidil-induced relaxation responses in tracheas isolated from LPS-and control groups (Table 1 and Figure 5).

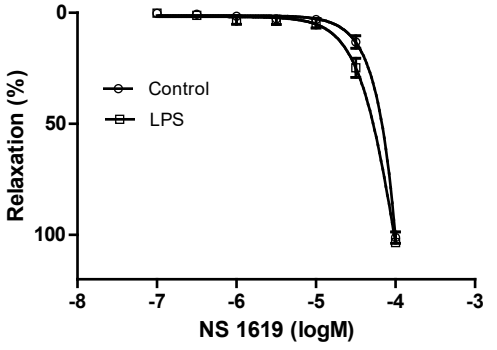

Figure 2. Effect of lipopolysaccharide (LPS) exposure on NS1619-induced relaxation in isolated mouse tracheal rings. Concentration-dependent relaxation responses to the large-conductance Ca^{2+}-activated potassium (BK_{Ca}) channel activator NS1619 were obtained in carbachol-precontracted tracheal rings isolated 48 h after intranasal administration of phosphate-buffered saline (control) or LPS. Relaxation is expressed as a percentage of the carbachol-induced precontraction. Data are presented as the mean ± SEM (n=6 per group). Statistical comparisons were performed using two-way ANOVA followed by Tukey's multiple-comparisons test

Table 1. The E_{max} values obtained from concentration-dependent relaxation responses of pinacidil, NS 1619, NS 309, and flupirtine in LPS and control groups

	Control		LPS	
	E_{max} (%)	n	E_{max} (%)	n
Pinacidil	96.58±6.35	**5**	100.83±1.37	**6**
NS 1619	101.29±2.53	**6**	103.57±1.02	**6**
NS 309	91.89±2.99	**6**	91.39±5.28	**6**
Flupirtine	76.68±7.21	**6**	85.79±7.60	**6**

E_{max}: Maximum relaxation response (%Carbachol pre-contraction)

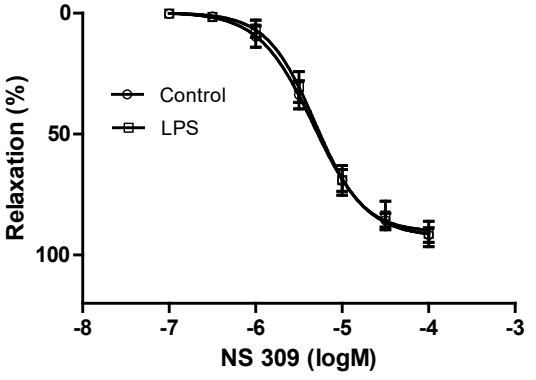

Figure 3. Effect of lipopolysaccharide (LPS) exposure on NS309-induced relaxation in isolated mouse tracheal rings. Concentration-dependent relaxation responses to the small- and intermediate-conductance Ca^{2+}-activated potassium ($K_{Ca2}/K_{Ca3.1}$) channel activator NS309 were obtained in carbachol-precontracted tracheal rings isolated 48 h after intranasal administration of phosphate-buffered saline (control) or LPS. Relaxation is expressed as a percentage of the carbachol-induced precontraction. Data are presented as the mean ± SEM (n=6 per group). Statistical comparisons were performed using two-way ANOVA followed by Tukey's multiple-comparisons test

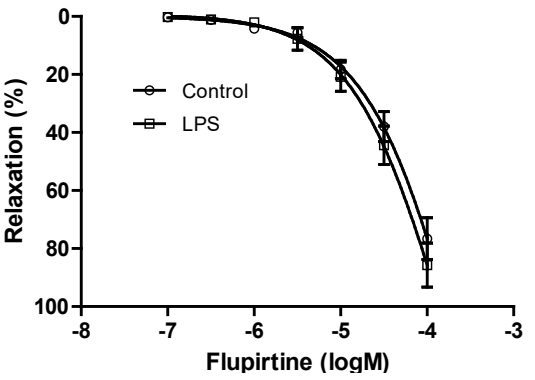

Figure 4. Effect of lipopolysaccharide (LPS) exposure on flupirtine-induced relaxation in isolated mouse tracheal rings. Concentration-dependent relaxation responses to the voltage-gated potassium channel Kv7 activator flupirtine were obtained in carbachol-precontracted tracheal rings isolated 48 h after intranasal administration of phosphate-buffered saline (control) or LPS. Relaxation is expressed as a percentage of the carbachol-induced precontraction. Data are presented as the mean ± SEM (n=6 per group). Statistical comparisons were performed using two-way ANOVA followed by Tukey's multiple-comparisons test

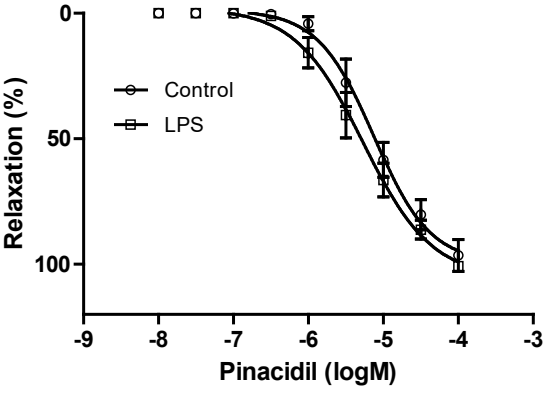

Figure 5. Effect of lipopolysaccharide (LPS) exposure on pinacidil-induced relaxation in isolated mouse tracheal rings. Concentration-dependent relaxation responses to the ATP-sensitive potassium (K_{ATP}) channel activator pinacidil were obtained in carbachol-precontracted tracheal rings isolated 48 h after intranasal administration of phosphate-buffered saline (control) or LPS. Relaxation is expressed as a percentage of the carbachol-induced precontraction. Data are presented as the mean ± SEM (control, n=5; LPS, n=6). Statistical comparisons were performed using two-way ANOVA followed by Tukey's multiple-comparisons test

The present study directly compared relaxation responses to isoprenaline and four pharmacological potassium channel activators—NS1619, NS309, flupirtine, and pinacidil-in carbachol-precontracted tracheal rings isolated from PBS-treated control mice and LPS-treated mice. Two principal findings have emerged. First, the four potassium channel activators produced comparable concentration-dependent relaxation in tracheal rings from PBS-treated mice. Second, LPS treatment attenuated isoprenaline-induced relaxation without significantly altering the responses to any of the potassium channel activators. This dissociation indicates that β_2-adrenoceptor-mediated and direct potassium channel-mediated relaxation differ in their susceptibility to LPS-induced airway inflammation. More specifically, the preserved responses to channel activators are consistent with the maintenance of the electromechanical mechanisms required for hyperpolarization-mediated relaxation, despite the impairment of conventional adrenoceptor signaling.

Airway inflammation is a central feature of asthma and chronic obstructive pulmonary disease (COPD) and contributes to airway hyperresponsiveness and impaired bronchodilator efficacy (Holgate, 2008; Varricchi, Brightling, Grainge, Lambrecht, & Chanez, 2024). LPS, a component of the outer membrane of Gram-negative bacteria, is also associated with environmental and occupational exposures linked to airway inflammation. Experimental LPS challenge reproduces several features of inflammatory airway disease, including innate immune activation, inflammatory mediator release, and increased airway responsiveness, and is therefore widely used to model environmentally induced inflammation and bacterial exacerbations (Kharitonov & Sjöbring, 2007; Starkhammar et al., 2012; 2014). Nevertheless, LPS is a predominantly acute innate immune stimulus and does not reproduce the full immunological and structural complexity of chronic asthma or COPD.

The attenuation of isoprenaline-induced relaxation following LPS exposure is consistent with previous evidence that inflammatory stimuli compromise ASM responsiveness to β-adrenoceptors. Exposure of guinea pig and rabbit airway tissues to pro-inflammatory cytokines, particularly tumor necrosis factor-α, reduces β-adrenoceptor-mediated relaxation through alterations in receptor-dependent and post-receptor signaling events (Hakonarson, Herrick, Serrano, & Grunstein, 1996; Wills-Karp et al., 1993). Cytokines can also impair adrenoceptor responsiveness through PKA- and prostaglandin E_2 receptor-dependent mechanisms, whereas Toll-like receptor activation can promote tachyphylaxis to agonists (Guo et al., 2005; Rahman et al., 2014). Thus, the diminished isoprenaline response observed in the present study may reflect impaired adrenoceptor coupling, altered cAMP–PKA signaling, increased production of inflammatory mediators, or a combination thereof. As these signaling components were not measured directly, the precise location of the functional defect could not be determined. Therefore, the findings should be interpreted as evidence of selective impairment of adrenoceptor-mediated relaxation, rather than definitive proof of receptor desensitization.

In contrast to isoprenaline, the relaxation induced by NS1619 and NS309 was preserved after LPS exposure. BK_{Ca} channels oppose muscarinic receptor-mediated depolarization and limit voltage-dependent Ca^{2+} entry, thereby restraining cholinergic contraction (Kume, Mikawa, Takagi, & Kotlikoff, 1995; Semenov, Wang, Herlihy, & Brenner, 2006). These channels may also contribute to β_2-adrenoceptor-mediated relaxation, as the inhibition of Ca^{2+}-activated K^+ currents attenuates isoprenaline-induced relaxation in airway preparations (Jones, Charette, Garcia, & Kaczorowski, 1990; Kume et al., 1995). The maintenance of NS1619-induced relaxation suggests that the functional pathway recruited by direct BK_{Ca} activation remained responsive after LPS exposure. This finding makes a generalized failure of BK_{Ca}-dependent hyperpolarization an unlikely explanation

for the reduced isoprenaline response in the present study study (Goldklang et al., 2013)

The preserved response to NS309 suggests that the K_{Ca2}/$K_{Ca3.1}$-sensitive relaxant pathway remains functional in this model. However, the cellular basis of this response requires careful interpretation of the results. $K_{Ca3.1}$ is more strongly associated with ASM proliferation and remodeling than with acute regulation of contractile tone, whereas K_{Ca2} channels expressed in non-muscle airway compartments may contribute to relaxation (Bradding & Wulff, 2009; Orfali et al., 2023). Furthermore, most disease-modifying evidence concerning $K_{Ca3.1}$ supports channel inhibition rather than its activation. In an OVA-induced asthma model, the $K_{Ca3.1}$ inhibitor TRAM-34 reduced eosinophilic inflammation, cytokine production, ASM remodeling, and airway hyperresponsiveness (Girodet et al., 2013). Therefore, acute activation and sustained inhibition of this channel family may have distinct consequences: activation of K_{Ca} conductance may facilitate short-term hyperpolarization, whereas chronic inhibition of $K_{Ca3.1}$ may suppress inflammatory cell activity and structural remodeling. Consequently, therapeutic implications depend on the channel subtype, cellular compartment, disease stage, and duration of intervention.

LPS exposure did not significantly affect flupirtine-induced relaxation, supporting the preservation of the functional mechanism engaged by K_v7. $_{Kv7}$ channels contribute to the resting membrane conductance of ASM, and their suppression by bronchoconstrictor agonists such as histamine promotes membrane depolarization and contraction (Brueggemann et al., 2012; Evseev et al., 2013; Haick et al., 2017; Mondéjar-Parreño et al., 2020). Conversely, pharmacological K_v7 activation restores the outward K^+ current, promotes hyperpolarization, and induces airway relaxation. Retigabine has also been reported to enhance formoterol-induced relaxation in rat airways, suggesting that K_v7 activation may complement adrenoceptor agonism and reduce the functional consequences of diminished agonist responsiveness (Brueggemann et al., 2014). The preservation of flupirtine-induced relaxation after LPS exposure supports $_{Kv7}$ channels as potential receptor-independent or adjunctive bronchodilator targets. Nevertheless, flupirtine is not selective for an individual K_v7 subtype and is unsuitable for clinical repurposing because of its adverse effect profile. The present findings consequently support the K_v7 pathway, rather than flupirtine itself, as a therapeutic candidate.

Pinacidil-induced relaxation was preserved after LPS exposure. K_{ATP} channels couple the cellular metabolic state to membrane excitability through Kir6.x pore-forming subunits and sulfonylurea receptor regulatory subunits (Aguilar-Bryan et al., 1998). Because inflammation is associated with oxidative and nitrosative stress and may alter cellular bioenergetics, K_{ATP} channel responsiveness could potentially be modified under inflammatory conditions (Ricciardolo et al., 2005). The maintained pinacidil response indicates that the functional pathway recruited by K_{ATP} opening remained sufficiently intact to produce relaxation in the LPS model used in the present study. This finding identifies K_{ATP} activation as a potential means of bypassing impaired β_2-adrenoceptor signaling. However, its clinical translation has been limited by the expression of related channels in vascular smooth muscle. Oral BRL 38227 produced little clinically useful bronchodilation in patients with asthma and frequently caused headaches, reflecting the difficulty of separating airway effects from systemic vasodilation (Kidney et al., 1993). Accordingly, future K_{ATP}-based strategies will require airway-restricted delivery or substantially improved tissue selectivity.

In addition to plasma membrane conductance, mitochondrial potassium channels may provide an additional link between inflammation, cellular metabolism, and airway responsiveness. Mitochondrial K^+ flux regulates the mitochondrial membrane potential, matrix volume, Ca^{2+} handling, ATP production, and reactive oxygen species generation. These

processes are frequently disrupted in inflammatory airway diseases and may influence ASM contractility, inflammatory signaling, survival, and remodeling. Therefore, mitochondria-targeted potassium channel modulators could offer effects distinct from the acute bronchodilation produced by plasma membrane channel activation. This possibility is particularly relevant to K_{ATP} pharmacology because pinacidil may influence both plasmalemmal and mitochondrial K_{ATP}-sensitive mechanisms, although the present organ bath experiments cannot distinguish between these sites of action.

The therapeutic potential of mitochondrial potassium channel modulation requires caution. The opening of mitochondrial channels should not be assumed to hyperpolarize the plasma membrane or directly relax ASM. Moreover, mitochondrial K_{ATP} channel activation has been reported to increase ASM cell proliferation through the phosphoinositide 3-kinase/protein kinase B (AKT) pathway in a rat model of asthma, suggesting that sustained activation could potentially promote airway remodeling (Gao, Sun, & Liu, 2021). Thus, mitochondria-targeted potassium channel openers cannot yet be regarded as established anti-inflammatory or bronchodilator agents. Future studies should distinguish the acute effects of these factors on airway tone from their longer-term effects on oxidative stress, inflammatory signaling, and ASM proliferation. Organelle-targeted compounds, direct assessment of mitochondrial membrane potential and respiration, and parallel evaluation of contractile and remodeling outcomes are required to determine whether mitochondrial potassium channels are beneficial or detrimental therapeutic targets in inflammatory airway disease.

Although NS1619, NS309, flupirtine, and pinacidil produced comparable relaxation profiles in control tissues, this functional similarity should not be interpreted as evidence that the four compounds or their target channels are mechanistically interchangeable. These agents differ substantially in terms of channel selectivity, potency, cellular distribution, and off-target pharmacology. At higher concentrations used in organ bath experiments, convergence on shared mechanisms, such as reduced Ca^{2+} entry or altered intracellular Ca^{2+} handling, may contribute to a common maximal relaxation. In particular, NS1619 may inhibit L-type Ca^{2+} channels independently of BK_{Ca} activation, and NS309 activates both K_{Ca2} and $K_{Ca3.1}$ channels; flupirtine is not Kv7-subtype selective, and pinacidil may exert effects beyond K_{ATP} opening. Consequently, the current results demonstrate preserved pharmacological responsiveness to these activators but do not establish that the expression or intrinsic activity of the corresponding channel proteins was unchanged by LPS.

This study has several limitations. It employed a single acute inflammatory model, one species, and proximal airway tissue; therefore, the findings may not extend directly to chronic allergic inflammation, COPD, or human small airways. Channel expression, membrane potential, intracellular Ca^{2+}, cAMP formation, and PKA activity were not measured. In addition, selective antagonists were not used to quantify the channel-dependent component of each response. Studies employing iberiotoxin or paxilline for BK_{Ca}, apamin and TRAM-34 for K_{Ca2} and $K_{Ca3.1}$, respectively, XE991 for K_v7, and glibenclamide for K_{ATP} would strengthen target attribution. Forskolin or membrane-permeable cAMP analogs could help determine whether the LPS-induced impairment occurs at the β2-adrenoceptor/Gαs interface or further downstream. Epithelium-denuded preparations would also clarify whether the preserved responses, particularly to NS309, originate from ASM or epithelium-dependent mechanisms.

CONCLUSION

To our knowledge, this is the first study to compare isoprenaline with activators of BK_{Ca}, K_{Ca2}/$K_{Ca3.1}$, K_v7, and K_{ATP} channels in the same airway preparation under both control and LPS-induced inflammatory conditions. The preservation of responses to all

four potassium channel activators, despite the attenuation of isoprenaline-induced relaxation, suggests that direct manipulation of membrane excitability may provide a route for bronchodilation when β2-adrenoceptor responsiveness is compromised. However, the historical limitations of systemic potassium channel openers indicate that clinical translation will require airway-selective delivery, improved channel and tissue subtype selectivity, and careful distinction between acute bronchodilator effects and longer-term consequences for inflammation and remodeling. Within these constraints, potassium channels, at both the plasma membrane and potentially the mitochondrial level, represent promising targets for developing receptor-independent or adjunctive therapies for inflammatory airway obstruction.

AUTHOR CONTRIBUTION STATEMENT

Hypothesis development: YKY and ISE; experimental work: YKY and TEB; manuscript drafting: YKY and TEB; manuscript review: TEB; statistical analysis, data analysis and interpretation, and literature review: YKY.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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