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Original research article

NO synthase inhibition attenuates EDHF-mediated relaxation induced by TRPV4 channel agonist GSK1016790A in the rat pulmonary artery: Role of TxA_2

M. Pule Addison, Thakur Uttam Singh *, Subhashree Parida, Soumen Choudhury, Jaya Kiran Kasa, Susanth V. Sukumaran, Sajad Ahmad Darzi, Kannan Kandasamy, Vishakha Singh, Dinesh Kumar, Santosh Kumar Mishra

Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar, India

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ABSTRACT

Background: The aim of the present study was to observe the concomitant activation of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) pathways by TRPV4 channel agonist GSK1016790A in the rat pulmonary artery and explore the mechanism by which NO synthase inhibition attenuates EDHF-mediated relaxation in endothelium-intact rat pulmonary artery.
Methods: Tension experiments were conducted on the pulmonary artery from male Wistar rats.
Results: TRPV4 channel agonist GSK1016790A (GSK) caused concentration-dependent relaxation (E_{max} 86.9 ± 4.6%; pD_2 8.7 ± 0.24) of the endothelium-intact rat pulmonary artery. Combined presence of apamin and TRAM-34 significantly attenuated the relaxation (E_{max} 61.1 ± 6.0%) to GSK. L-NAME (100 μM) significantly attenuated (8.2 ± 2.9%) the relaxation response to GSK that was resistant to apamin plus TRAM-34. However, presence of ICI192605 or furegrelate alongwith L-NAME revealed the GSK-mediated EDHF-response (E_{max} of 28.5 ± 5.2%; E_{max} 24.5 ± 4.3%) in this vessel, respectively. Further, these two TxA_2 modulators (ICI/furegrelate) alongwith L-NAME had no effect on SNP-induced endothelium-independent relaxation in comparison to L-NAME alone. This EDHF-mediated relaxation was sensitive to inhibition by K^+ channel blockers apamin and TRAM-34 or 60 mM K^+ depolarizing solution. Further, combined presence of apamin and TRAM-34 in U46619 pre-contracted pulmonary arterial rings significantly reduced the maximal relaxation (E_{max} 71.6 ± 6.9%) elicited by GSK, but had no effect on the pD_2 (8.1 ± 0.03) of the TRPV4 channel agonist in comparison to controls (E_{max}, 92.4 ± 4.3% and pD_2, 8.3 ± 0.06).
Conclusion: The present study suggests that NO and EDHF are released concomitantly and NO synthase inhibition attenuates GSK-induced EDHF response through thromboxane pathway in the rat pulmonary artery.

Introduction

Endothelial dysfunction is considered to play an important role in pulmonary hypertension [1]. Therefore, it is of considerable interest to understand the mechanisms underlying endothelium-dependent regulation of pulmonary arterial tone in health and diseases. The endothelium in pulmonary blood vessels regulates the vascular tone by release of several vasoactive factors that include both vasodilators such as nitric oxide, endothelium-derived hyperpolarizing factors (EDHF) and prostacyclin as well as vasoconstrictors like thromboxane A_2, endothelin, leukotrienes and superoxide anions [2]. In the rat pulmonary artery, it has been

Abbreviations: ACh, acetylcholine; ANOVA, analysis of variance; EDHF, endothelium-derived hyperpolarizing factor; GSK, GSK1016790A; ICI192605, [4-(Z)-6-(2-0-chlrophenyl-4-Ohydroxyphenyl-1,3-dioxan-cis5-yl) hexenoic acid]; IK_{Ca} (K_{Ca}3.1), intermediate conductance potassium channel; L-NAME, N^G-nitro-L-arginine methyl ester; MKHS, modified Krebs–Henseleit solution; NO, nitric oxide; NOS, nitric oxide synthase; PE, phenylephrine; PKG, protein kinase G; SK_{Ca} (K_{Ca}2.3), small conductance potassium channel; SNP, sodium nitroprusside; TRAM-34, 1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole; TRPV4, transient receptor potential vanilloid 4; TP, thromboxane/prostanoid receptor.

* Corresponding author.
E-mail address: tusingh80@gmail.com (T.U. Singh).

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shown that nitric oxide and EDHF are the two predominant vasodilators mediating endothelium-dependent relaxation [3–6]. Nitric oxide (NO) and EDHF operate simultaneously in middle cerebral artery and various other vasculatures [7–9]. There are various substances which are responsible for release of NO and EDHF like acetylcholine in the canine [10] and rat pulmonary arteries [3,4] bradykinin in bovine pulmonary supernumerary arteries [11] and pulmonary resistance arteries of the newborn piglet [12]. However, concomitant release of NO and EDHF by TRPV4 channel agonist GSK1016790A is not clear in the rat pulmonary vasculature.

IK_{Ca} and SK_{Ca} channels are responsible for EDHF- mediated relaxation in endothelium of pulmonary arteries and bronchiolar epithelium of the rat [13]. The loss of the EDHF response may be primarily responsible for the endothelial dysfunction in sepsis, and its restoration by a selective iNOS inhibitor may improve pulmonary vasodilation [5].

Previously, it has been reported that GSK-induced relaxation was negligible in presence of L-NAME in the rat pulmonary artery [6]. However, an amplified relaxation response was observed in presence of indomethacin along with L-NAME which had been defined as EDHF [6]. A previous report demonstrated that exogenously applied NO attenuated the EDHF response in rabbit carotid and porcine coronary arteries [14] On the other hand, one component, SK_{Ca} channel of EDHF response was inhibited by blockage of nitric oxide synthase (NOS) in middle cerebral artery of rat [15]. A previous report demonstrated that TxA_2 receptor stimulation leads to progressive loss of smooth muscle hyperpolarization due to EDHF which occurs due to inactivation of the endothelial SK_{Ca} in the rat isolated mesenteric artery [16]. TxA_2 synthesis could be inhibited with NO by interacting with heme active site of thromboxane synthase enzyme [17] and further a report stated that NO desensitizes thromboxane receptors through PKG signaling pathway [18]. Activation of endothelial and epithelial $K_{Ca}2.3$ channels leads to relaxation in small pulmonary arteries and bronchioles in human [19]. However, in current study we have tried to explore the interaction between NO and EDHF as it is poorly understood. Some reports also suggest that NO inhibits EDHF-response in various blood vessels but it is not studied in rat pulmonary artery, moreover, its mechanism of action still remains unclear. Therefore, the first objective of the present study was to study the concomitant activation of NO and EDHF pathways by TRPV4 channel agonist GSK1016790A in rat pulmonary artery. The second objective was to explore the mechanism by which NO synthase inhibition attenuates EDHF-mediated relaxation in endothelium-intact rat pulmonary artery.

Materials and methods

Animals

Healthy adult male Wistar rats (150–200 g) were procured from the Laboratory Animal Resource Section, Indian Veterinary Research Institute, Izatnagar, India. Animals were kept for acclimatization for a period of seven days before conduction of experiments. All protocols, employed in this study, were approved by the Institutional Animal Ethics Committee, Indian Veterinary Research Institute, Izatnagar.

Tension recording

The animals were killed by excising the abdominal aorta under urethane (1.2 g/kg body weight intraperitoneally) anesthesia. Heart and lungs *enbloc* were removed and transferred to ice cold Modified Krebs–Henseleit solution (MKHS) of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 11.9, KH_2PO_4 1.2 and D-glucose 11.1 (pH 7.4). Both the branches of the main pulmonary artery (right and left) were dissected carefully and cleaned of adhering connective tissues under a dissecting microscope. These pulmonary artery rings were used for further study. The rat pulmonary arterial rings were mounted on two stainless steel hooks and suspended in 10 ml organ bath containing MKHS, maintained at 37 °C. Further, pulmonary artery rings were continuously aerated with medical gas (21% O_2 + 5% CO_2 + 74% N_2) mixture. One g passive tension was applied during the equilibration period of 90 min and the bath solution was changed every 15 min. Tension was recorded using a high sensitivity isometric force transducer and stored in a computer using Lab Chart version 5.4.1 software program (Powerlab, AD Instruments, Bella Vista, NSW, Australia) for further analysis.

After the completion of equilibration period, tissue viability was checked by recording the contraction to 80 mM K^+ depolarizing solution. Concentration–response curves to relaxants (GSK and SNP) were elicited in arterial segments pre-contracted with a submaximal concentration of phenylephrine (0.1–1 μM) or U46619 (10–100 nM) to achieve near identical pre-contraction levels. 1 μM of ACh was added at the plateau of phenylephrine contraction to examine the endothelial integrity. If the relaxant response to 1 μM ACh was more than 80%, it was considered endothelium-intact pulmonary artery. L-NAME pretreatment sensitized the pre-contractions to phenylephrine before eliciting relaxation responses to different vasodilators. Hence, an appropriate concentration of phenylephrine, as stated above, was used to achieve matching contraction level of the arterial segments before eliciting relaxations to vasodilators [6].

Experimental protocols

Concomitant activation of NO and EDHF pathways by TRPV4 channel agonist GSK1016790A in the rat pulmonary artery

To examine the concomitant release of NO and EDHF by TRPV4 channel agonist GSK1016790A (GSK), a concentration response curve to GSK was developed in combined presence of 100 nM apamin and 1 μM TRAM-34. GSK (10^{-10}–10^{-7} M), added cumulatively at an increment of 0.5 log unit, caused concentration-dependent relaxation in endothelium-intact pulmonary artery rings pre-contracted with 0.1–1 μM phenylephrine. Apart from this, a dose-response to GSK was elicited in combined presence of L-NAME, apamin and TRAM-34.

Effect of L-NAME and ICI192605 (ICI) (a thromboxane receptor antagonist) or furegrelate (thromboxane synthase blocker) on SNP-induced relaxation in the pulmonary artery

To confirm the specificity of EDHF-mediated GSK-induced relaxation, a concentration-response curve to SNP (10^{-11}–10^{-5} M) was elicited in pulmonary arterial rings pretreated for 30 min with 100 μM L-NAME and 100 μM L-NAME plus 10 μM ICI/10 μM furegrelate.

To elucidate the L-NAME resistant GSK-induced relaxation modulation by ICI192605 or furegrelate in the rat pulmonary artery

In order to assess the modulatory effect of vasocontractile substances in endothelium like prostanoids, the pulmonary arterial rings were pretreated with either ICI (a thromboxane receptor blocker) or furegrelate (a thromboxane synthase inhibitor). The contribution of EDHF pathway to GSK (10^{-10}–10^{-7} M)-induced relaxation was evaluated by eliciting concentration-dependent relaxation to the TRPV4 channel agonist on phenylephrine-contracted pulmonary artery rings pre-treated with 100 μM L-NAME plus 10 μM ICI/10 μM furegrelate for 30 min.