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Evaluation of the component's contribution in endothelium-dependent acetylcholine-induced relaxation of the rat aorta

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The regulation of rat aorta vascular tone involves various factors, including endothelium-derived hyperpolarization factor (EDHF), nitric oxide (NO), prostaglandins, and sensory nerves. While these elements can function independently, their pathways intersect at various points, complicating the assessment of their individual contributions. The aim of this study was to establish the numerical contributions of EDHF, NO, prostaglandins, and also the effect of the sensory nerve on acetylcholine-induced relaxation on the background of phenylephrine preconstriction using contraction and relaxation measurements in Wistar rat thoracic aorta. EDHF, whose action is mediated through potassium channels, emerges as a crucial regulator. Blockage of inward rectifier potassium (KIR) channels integral to EDHF significantly abolishes 50% of the relaxation amplitude in comparison to control conditions. Endothelial TRPV4 channel, exhibiting a fine-tuning role, contributes to a 25% reduction in the amplitude of acetylcholine-induced relaxation in comparison to control relaxation. NO demonstrates its vasodilatory prowess, with NO blockage eliminating 77% of the residual relaxation effect after KIR blockage. Blockage of prostaglandin functions, modulated by cyclooxygenase 1, reduces relaxation by 44% in comparison to control relaxation. Desensitization of sensory nerves with capsaicin, shows a minor yet significant role, in the reduction of acetylcholine-induced relaxation amplitude by 10%. In conclusion, we established that the main element of acetylcholine-induced relaxation is EDHF with approximately 50% of relaxation amplitude depending on it.
Key words: aorta; endothelium; endothelium dependent hyperpolarizing factor; NO; vasodilatation; TRPV4.

INTRODUCTION

The aorta, being the largest vessel in the body, undergoes regulation by numerous components, both ion channels and chemicals. These components may intersect or act in opposing ways, adding complexity to the regulation of smooth muscle aortic tone. While many factors involved in aortic tone regulation have been studied extensively, the quantitative contributions of each factor to acetylcholine-induced relaxation remain unknown to date.

The components involved in acetylcholine-induced aortic relaxation can be categorized into several distinct groups, each associated with different signaling pathways. These groups encompass endothelium-derived hyperpolarizing factor (EDHF), nitric oxide (NO), prostaglandins, and sensory nerves. These components play crucial roles in modulating the complex process of aortic relaxation, and their interactions form a comprehensive network of signaling mechanisms [1]. Our study seeks to unravel the quantitative contributions of each of these components to acetylcholine-induced relaxation on the background of phenylephrine preconstriction, shedding light on their intricate interplay.

EDHF stands out as a predominant mechanism by which endothelial cells regulate the relaxation of the vascular wall. Several hypotheses that explain the nature of EDHF exist, while one of the most common ones connects it to the direct impact on potassium channels. This intricate process involves the

activation of calcium-activated potassium channels, specifically small conductance (SK), intermediate conductance (IK), and big conductance (BK). These channels are triggered by intracellular calcium spikes. As a result, direct activation of inward rectifier potassium (KIR) channels in smooth muscle cells (SMC) and membrane hyperpolarization due to a change in potassium concentration generated by outward potassium flow due to SK/IK/BK channels of endothelial cells [2, 3]. Subsequently, this hyperpolarization can propagate through mioendothelial gap junctions and/or via KIR on SMC, thereby reducing the probability of SMC contraction [4, 5].

The initial activation of potassium channels on endothelial cells involves various factors, with one key player being transient receptor potential vaniloid subfamily (TRPV) channels. TRPV channels are recognized as sensitive and fine-tuning regulators of multiple processes, encompassing thermal sensitivity, ligand binding, mechanosensitivity, and voltage gating. Notably, TRPV4 channels are often colocalized with IK/SK channels on the membrane of endothelium cells, suggesting that their activation should trigger potassium channel activation through a calcium-dependent mechanism [6]. This cascade of events, in turn, leads to the release of EDHF [7, 8].

NO emerges as another formidable regulator of vascular tone, particularly exerting influence on basal tone while possessing robust vasodilatory properties. Within the vascular wall under normal conditions, NO is primarily synthesized by endothelial Ca^{2+}/calmodulin-dependent NO synthase (eNOS) [9]. Its mode of action involves paracrine signaling, where it acts on nearby cells, inducing vasodilation in SMCs through the activation of guanylate cyclase. This activation results in the production of cyclic guanosine monophosphate (cGMP), initiating downstream regulatory processes. Notably, these processes include the inactivation of calcium channels and the phosphorylation of the myosin light chain, collectively contributing to the relaxation of smooth muscle cells [10].

Prostaglandins (prostacyclin) are recognized as potent vasodilators produced by cyclooxygenase 1. They typically act through the protein kinase A pathway. Similar to NO, prostaglandins induce vasodilation by phosphorylating the myosin light chain and inactivating calcium channels. This process involves hyperpolarization caused by the activation of potassium channels triggered by phosphorylation [11].

The final component is associated with sensory nerve endings through a noncholinergic nonadrenergic mechanism. This system can release potent vasodilators such as calcitonin gene-related peptide and substance P, which can act through NO-dependent or KIR-dependent pathways, inducing vasodilation in the aorta [11, 12].

The aim of our study was to elucidate the numerical contributions of various components to acetylcholine-induced relaxation against the background of previous vasoconstriction by phenylephrine and investigate their intersections.

METHODS

In our study, rats were used, following animal protocols per EU Directive 2010/63/EU for animal experiments (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm). The Bioethics Committee of the Bogomoletz Institute of Physiology approved the experimental protocol under Permission No. 2/17 on 05.09.2017. Rats were bred, housed, and cared for in the BIPh vivarium, with strict measures to minimize potential suffering.

Male Wistar rats weighing 200-250 g, were used in the experiments. Their aortas were carefully dissected from the aortic arch to the renal arteries under stereo-microscopic control, removing surrounding tissues. The aortas were then cut into segments 3-4 mm in length. For recording contraction and relaxation, segments were suspended in a Krebs solution-filled chamber at a constant flow rate of 6 ml

per minute. Each segment was secured with hooks-one attached to the chamber walls, and the other to a self-made tension sensor. Mechanical responses, measured isometrically, were digitized using Axon DigiData 1200 and displayed on a computer screen via Clampex 8.0.

The Krebs solution comprised (mmol/l): NaCl - 120, KCl - 5, $CaCl_2$ - 2, MgSO4 - 1, glucose -5, and Hepes - 7, balanced around pH 7.35 at 37°C.

Strips equilibrated in normal Krebs solution at 1 mN basal tension for 1 h before measurements. The contraction was induced by applying 10 μmol/l phenylephrine, and submaximal relaxation was achieved with 1 μmol/l acetylcholine, following a dose-effect curve built during experiments. Data acquisition used Clampex software, with subsequent analysis in Clampfit ("Axon Instruments", USA).

Chemicals used in the study included phenylephrine hydrochloride, acetylcholine chloride, barium chloride (non-specific inhibitor of KIR channels), ouabain octahydrate (specific inhibitor of Na^+/K^+ pump), NG-Nitro-L-arginine (non-specific inhibitor of NO-synthase), and 18α-glycyrrhetinic acid (specific inhibitor of gap junction), and 2-methyl-1-(3-morpholinopropyl)-5-phenyl-N-(3-(trifluoromethyl)phenyl)-1H-pyrrole-3-carboxamide (specific inhibitor of TRPV4 channels) ("Sigma-Aldrich", USA). Compounds were added directly to the experimental Krebs solution for desired working concentrations (μmol/l): phenylephrine -10, acetylcholine - 1, barium chloride - 100, ouabain - 40, 18α-glycyrrhetinic acid (18GA) dissolved in DMSO - 10, NG-Nitro-L-arginine(NG-arg) - 10, 2-methyl-1-(3-morpholinopropyl)-5-phenyl-N-(3-(trifluoromethyl)phenyl)-1H-pyrrole-3-carboxamide(HC-067047) (dissolved in DMSO) 1. Control experiments confirmed that up to 0.1% DMSO did not impact aorta ring contractility.

Statistical analysis of aorta relaxation data. The analysis commenced with the use of Clampfit software to extract numerical values of tensile force in mN. Amplitudes were determined by subtracting the minimum relaxation value from the maximum contraction value during the respective periods and then dividing the result by the basal tone. The formula used for normalizing the amplitude was:

$$normalized\ amplitude = \frac{(maximum\ contraction - minimum\ relaxation)}{basal\ tone}$$

To illustrate the relative contribution of the element in whole acetylcholine-induced relaxation normalized percentage reductions were calculated where we took normalized amplitude of control recording as 100%, and then all percentage reductions were calculated using the next formula:

$$percentage\ reduction = \frac{normalized\ amplitude\ after\ specific\ chemical}{normalized\ amplitude\ in\ control} \cdot 100$$

Then average of the percentage change was calculated for the given experimental condition. All numerical data is presented as a mean percentage of normalized amplitudes ± standard deviation. Subsequently, the analysis involved the comparison of normalized amplitude values across different segments to confirm the statistical significance of the difference in amplitudes. When comparing two groups, the Welch 2-sample test was applied, considering unequal variances and different sample sizes between groups. For comparisons involving more than two groups, Welch ANOVA was employed, with the Holm adjustment for multiple comparisons. Probabilities less than 5% were deemed statistically significant ($P < 0.05$). All statistical analyses were conducted using the R programming language [13]. Sample sizes were estimated using power analysis to enhance the statistical power of the chosen tests. The graphical representation includes violin plots showing normalized amplitudes before percentage calculations with pairwise comparisons, illustrating test values and P-values for better visualization and interpretation.

RESULTS AND DISCUSSION

Role of KIR channels in acetylcholine-induced relaxation. Our results indicate that, after a 20-min preapplication of Ba^{2+} and ouabain, the amplitude of acetylcholine-induced relaxation under phenylephrine pre-constricted conditions decreased by 50 ± 8% (n = 37) compared to the control relaxation (Fig. 1). This highlights the significant involvement of KIR channels in the regulation of aortic SMC function, accounting for approximately 50% of acetylcholine-induced relaxation.

The relative contribution of mioendothelial contacts and SMC-membrane in the propagation of EDHF. To quantify the contribution of EDHF in acetylcholine-induced relaxation, we aimed to investigate the importance of gap junctions. Selectively blocking these junctions by 18-GA, our findings revealed that acetylcholine-induced relaxation decreased by 50 ± 9% (n = 32) compared to the control scenario where only KIR channels were inhibited, while we did not show any significant difference with the only 18-GA scenario (Fig. 1). This suggests that a substantial portion of EDHF acts directly through gap junctions, while another fraction (~50%) is associated with KIR channels on the smooth muscle cell membrane.

The proportion of nitric oxide and KIR channels contribution in aortic acetylcholine-induced relaxation. To delineate the role of NO in acetylcholine-induced relaxation, we inhibited endothelial NO synthase activity with NG-arg, and to remove the contribution to the relaxation of KIR channels we added Ba^{2+} together with oubaine. The outcome demonstrated a substantial reduction, with relaxation being 77 ± 12% (n = 21) smaller than the control scenario which includes only barium and oubaine (Fig. 2). This underscores the pivotal role of NO, indicating that it plays a predominant role in acetylcholine-induced relaxation against the background of previous vasoconstriction by phenylephrine.

TRPV4 role in acetylcholine-induced relaxation. To study the effects of endothelial TRPV4 which is known to be connected with the potassium component of EDHF functions, we tried to specifically block the function of this channel. Subsequently, we demonstrated that the inhibition of the TRPV4 channel with

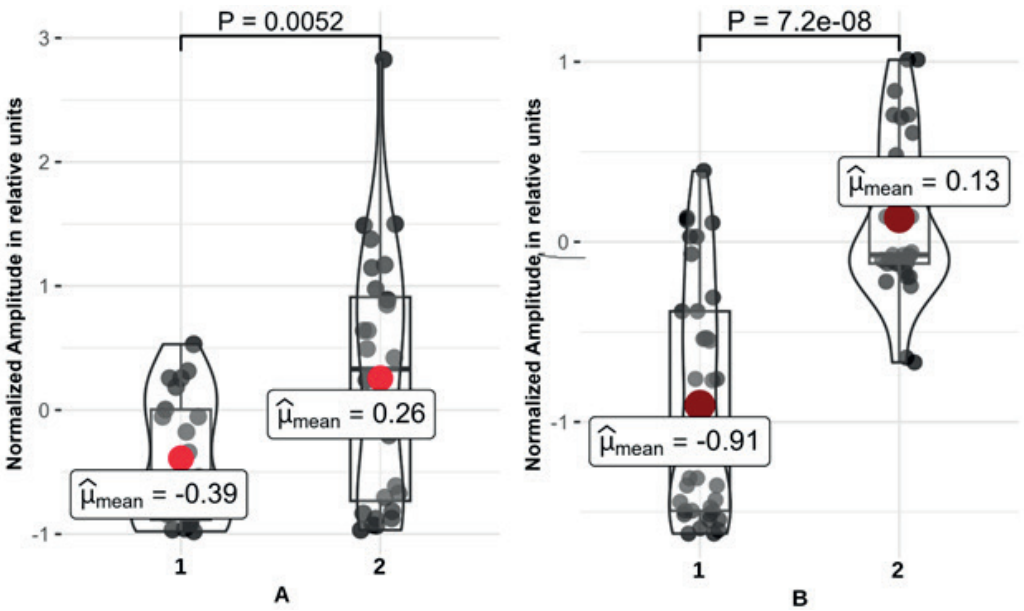

Fig. 1. Difference between amplitudes of acetylcholine-induced relaxation in control (A1, B1), and after Ba^{2+} and oubaine application (A2) and after Ba^{2+} and oubaine and 18-GA application (B2)

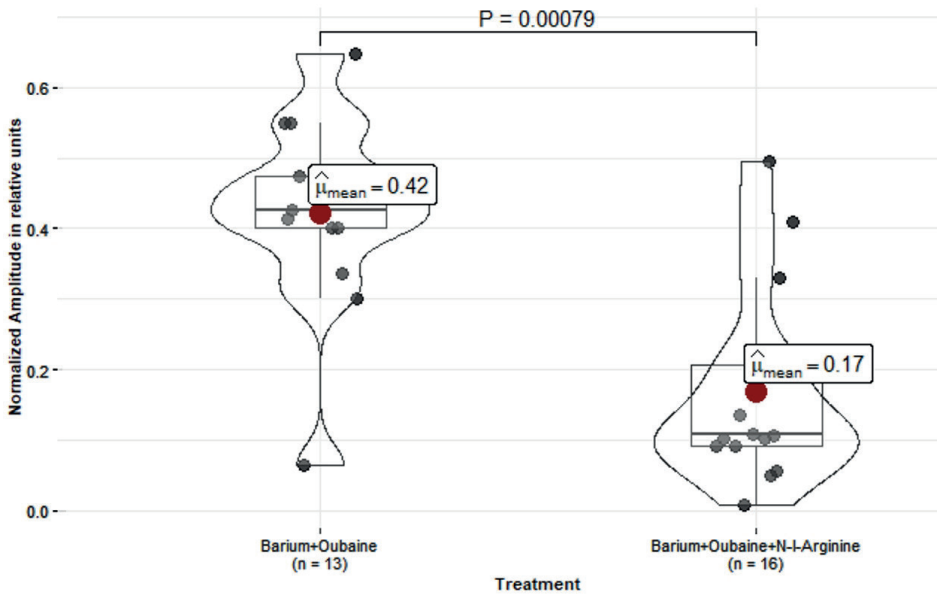

Fig. 2. Difference between the amplitudes of acetylcholine-induced relaxation in control and after preapplication of Ba^{2+} and oubaine and after Ba^{2+}, oubaine, and N^G-L-arginine preapplication

a selective inhibitor of TRPV4 HC-067047 resulted in a reduction in the amplitudes of acetylcholine-induced relaxation under phenylephrine preconstriction by 25 ± 9% (n = 19; Fig. 3). This observation underscores the significance of the activity of TRPV4 in the regulation of aortic relaxation, implicating its association with endothelial IK/SK channels and, consequently, EDHF.

Another way through which TRPV4 contributes to the regulation of vascular tone is via the myoendothelial feedback effect [13-15].

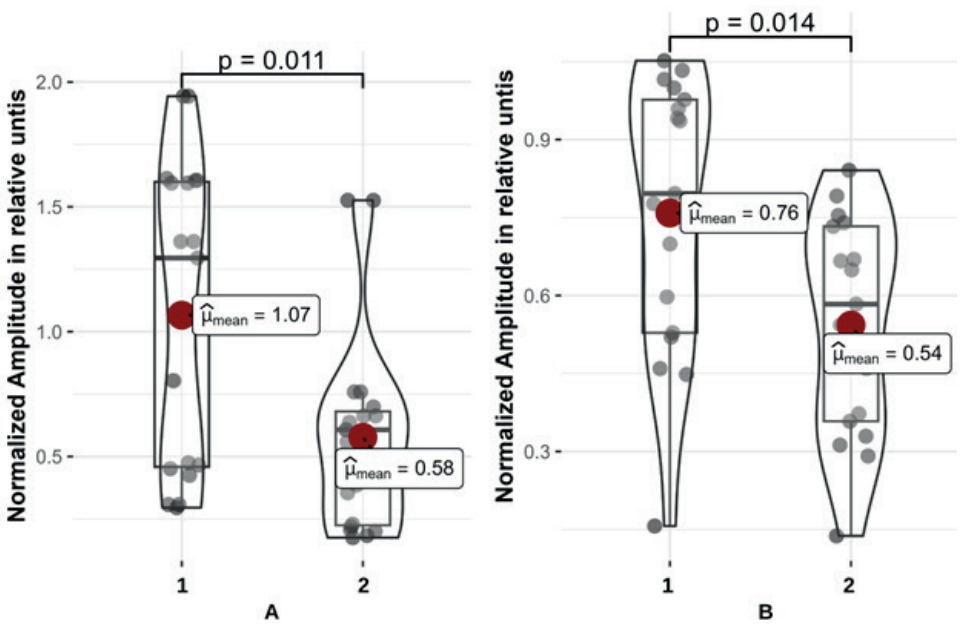

Fig. 3. Comparison of acetylcholine-induced relaxation amplitudes in control (A1, B1) and after HC-067047 and indomethacin preapplication (A2, B2)

We have shown this effect on the peaks of phenylephrine contractions and can be quantified by calculating the difference between the peaks of phenylephrine-induced contractions. Notably, this myoendothelial feedback effect is nullified by the application of HC-067047 for 20 min. In control experiments, this effect was approximated to constitute 10 ± 8% (n = 20) of the control acetylcholine-induced relaxation on phenylephrine-induced preconstriction.

Role of prostaglandins in acetylcholine-induced relaxation. The subsequent element of acetylcholine-induced endothelium-dependent relaxation under phenylephrine preconstriction is linked to prostaglandin signaling through the PKA signaling pathway, akin to the NO pathway. Non-selective inhibition of cyclooxygenase 1/2 by indomethacin unveiled that the amplitude of acetylcholine-induced endothelium-dependent relaxation under phenylephrine preconstriction was approximately 44 ± 14% (n = 20) smaller than in the control scenario (Fig. 3).

Contribution of sensory innervation to acetylcholine-induced aortic relaxation. Our results suggest that the remaining component 10 ± 4% (n = 50) of acetylcholine-induced relaxation under phenylephrine preconstriction can be ascribed to K_{ATP} channels on SMC activated by calcitonin gene-related peptide, released from the sensory nerves endings after activation of acetylcholine receptors, and to NO signaling pathway in the endothelial cells. Sensory denervation of these nerve endings through preconditioning with 1 μmol/l of capsaicin for 20 min, combined with subsequent blockade of NO synthase and KIR channels, led to a diminished amplitude of acetylcholine-induced relaxation under phenylephrine preconstriction compared to the scenario where only NO synthase and KIR channels were blocked, effectively eliminating that 10% of relaxation (n = 50; Fig. 4). To confirm the role of K_{ATP} channels in acetylcholine-induced aortic relaxation we employed the specific inhibitor of these channels glibenclamide, and observed the same 10% reduction (n = 11) in relaxation amplitude.

The outcomes of our investigation reveal that acetylcholine-induced relaxation in rat aorta on the background of preconstriction by phenylephrine is a multifaceted phenomenon comprised of distinct components with varying contributions to the process. The significance

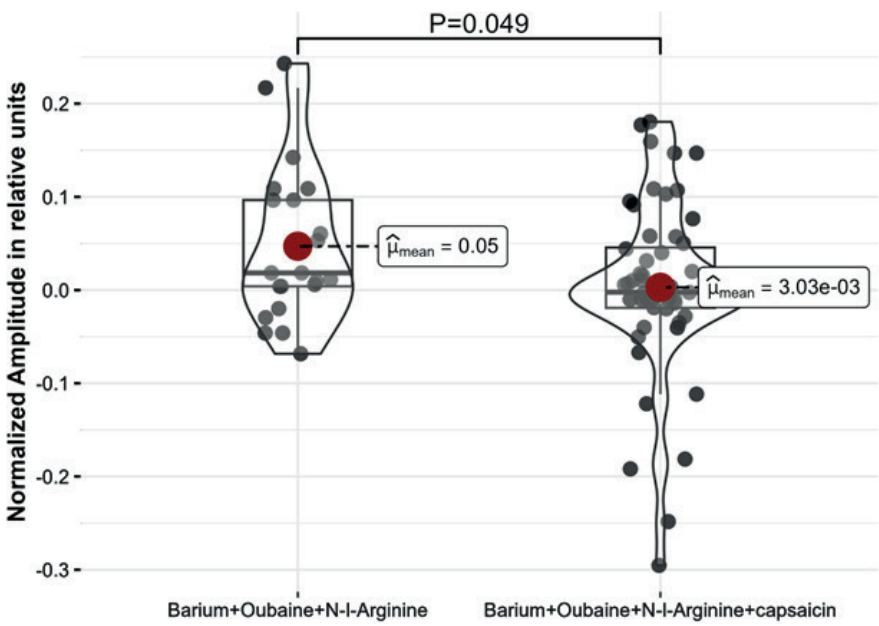

Fig. 4. Comparison of acetylcholine-induced relaxation amplitudes after application of Ba^{2+}, oubaine with N^G-L-arginine, and after preapplication of Ba^{2+}, oubaine, N^G-L-arginine, and capsaicin

of comprehending the contributions of these factors is paramount, given the crucial role of the aorta in supplying blood throughout the body and buffering pressure spikes to protect smaller vessels from rupture.

The foremost regulator of rat aorta basal tone is NO, acting through guanylate cyclase pathways. Our results indicate that NO blockage eliminates 38 ± 12% of acetylcholine-induced relaxation, underscoring its significant role in this process.

In addition to NO, EDHF exerts an incremental effect on vessel basal tone and plays a substantial role in acetylcholine-induced relaxation. While EDHF can be connected to different factors such as NO and H_2S which cause hyperpolarisation through indirect activation of potassium channels by secondary intracellular messengers [16, 17], in this study we focused on the direct activation of the potassium component of EDHF. Blockage of KIR channels, key players in EDHF propagation, abolishes 50 ± 8% of relaxation amplitude. EDHF influences smooth muscle cells through various mechanisms, including direct potassium currents through gap junctions between endothelial cells and SMC [18]. Our data demonstrates that 50 ± 12% of the EDHF effect is mediated by gap junctions, while the remaining portion acts via KIR channels on SMC.

Prostaglandins, another group of signaling molecules, are involved in acetylcholine-induced relaxation through the activation of protein kinase A and its downstream signaling pathway [19]. Our findings show that inhibiting prostaglandins through nonselective blockage of cyclooxygenase 1/2 reduces the amplitude of acetylcholine-induced relaxation by 44 ± 14%.

The last identified component of acetylcholine-induced relaxation in the aorta is associated with K_{ATP} channels and NO synthase regulated by the sensory nerves. Sensory nerve endings release substance P and calcitonin gene-related peptides, acting as nonadrenergic-noncholinergic vasodilators [11, 20, 21]. Our data indicates that capsaicin pretreatment, inducing sensory nerve desensitization, eliminates the last 10 ± 4% of acetylcholine-induced relaxation amplitude, demonstrating a small but significant effect on this phenomenon.

Furthermore, our findings indicate that functions of TRPV4 contribute to 25 ± 9% of acetylcholine-induced relaxation, exerting influence through both EDHF and NO functions [22]. Additionally, basal activity of TRPV4 has been implicated in myoendothelial feedback during vasoconstriction by phenylephrine, underscoring its intricate and fine-tuning role in aortic regulation [14]. This phenomenon was crucial for evaluating the role of TRPV4 in the involvement of the EDHF signaling pathway and understanding its effect on the regulation of vascular tone.

The challenge in summing all components to 100% can be attributed to the interactions and intersections between different pathways. Notably, the inhibition of cyclooxygenase 1/2 by indomethacin has broader implications than solely affecting the prostaglandin signaling pathway. Previous research has demonstrated that indomethacin, while not impacting the expression levels of iNOS or eNOS, still inhibits their activity through hemoxygenase blockage. This dual effect implies that indomethacin application will not only the prostaglandin pathway but also the NO function. Consequently, a portion of acetylcholine-induced relaxation inhibited by indomethacin will encompass contributions from both the NO and prostaglandin components [23, 24]. This phenomenon requires additional investigations in future studies to separate the effect of prostaglandins from NO effects.

CONCLUSIONS

In summary, we quantified the effects of different components of acetylcholine-induced relaxation of isolated aortic strips precontracted with phenylephrine, demonstrating that 50% of the effect relies on EDHF, 40% on prostaglandins, 10% is mediated by sensory nerves, while 30% can be explained by NO signaling pathway.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

О.Р. Меженський, І.Б. Філіппов

ВИЗНАЧЕНЯ ВНЕСКУ КОМПОНЕНТІВ ЕНДОТЕЛІАЛЬНОГО ПОХОДЖЕННЯ В АЦЕТИЛХОЛІНОВЕ РОЗСЛАБЛЕННЯ АОРТИ ЩУРА

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Регуляція судинного тонусу аорти щура включає такі різноманітні фактори , як ендотелійзалежний гіперполяризуючий фактор, оксид азоту, простагландини та нервові закінчення. Хоча ці елементи здатні функціонувати незалежно їхні сигнальні шляхи можуть перетинатися чи діяти в протилежних напрямках ускладнюючи визначення індивідуального внеску кожного з них. Мета нашої роботи – визначення кількісного внеску ендотелійзалежного гіперполяризуючого фактора, оксиду азоту, простагландинів та додаткового ефекту сенсорних нервів у ацетилхолінове розслаблення при фенілефриновому скороченні, використовуючи тензитометричний метод на грудній аорті щурів лінії Вістар. Ендотелійзалежний гіперполяризуючий фактор, модульований калієвими каналами, відіграє роль ключового регулятора у цьому процесі. Блокада калієвих каналів вхідного випрямлення, суттєво зменшувала амплітуду ацетилхолінового розслаблення на 50 ± 8% (n = 37). Ендотеліальний TRPV4 канал регулює тонус судинної стінки та відповідає за 25 ± 9% (n = 19) амплітуди ацетилхолінового розслаблення. Була показана суттєва вазодилатуюча дія оксиду азоту. Інгібування функції NO-синтази разом з блокадою калієвих каналів вхідного випрямлення зменшувала амплітуду ацетилхолінового розслаблення на 77 ± 12% (n = 21),а активності циклооксигенази 1,2 – на 44 ± 14% (n = 20). При десенситизації сенсорних нервів капсаїцином спостерігався невелике зменшення цього показника (10 ± 4%; n = 50). Таким чином, ми визначили, що основним елементом ацетилхолінового розслаблення є ендотелійзалежний гіперполяризуючий фактор, що вносить близько 50% у амплітуду ацетилхолінового розслаблення.

Ключові слова: аорта; ендотелій; ендотелійзалежний гіперполяризуючий фактор; NO; розслаблення; TRPV4.

REFERENCES

1. Quyyumi AA, Ozkor MA. Endothelium-derived hyperpolarizing factor and vascular function. Cardiol Res Pract. 2011;156: 14, 6.
2. Triggle C, Ceroni L, Jiang Y, Wiehler W, Ellis A, Ding H. Connexin, BK, IK, and SK expression and EDHF in mouse mesenteric vasculature. FASEB J. 2006 ; 20:A1175.
3. Jackson WF. Boosting the signal: Endothelial inward rectifier K^+ channels. Microcirculation. 2017; 24: 3.
4. Schmidt K, de Wit C. Endothelium-derived hyperpolarizing factor and myoendothelial coupling: the *in vivo* perspective. Front Physiol. 2020;11:602930.
5. Li L, Wu J, Jiang C. Differential expression of Kir6.1 and SUR2B mRNAs in the vasculature of various tissues in rats. J Membr Biol. 2003 196:61-9.
6. Lin MT, Jian MY, Taylor MS, Cioffi DL, Yap FC, Liedtke W, et al. Functional coupling of TRPV4, IK, and SK channels contributes to Ca^{2+}-dependent endothelial injury in rodent lung. Pulm Circ. 2015;5:279-90.
7. Huang J, Zhang H, Tan X, Hu M, Shen B. Exercise restores impaired endothelium-derived hyperpolarizing factor-mediated vasodilation in aged rat aortic arteries via the TRPV4-KCa2.3 signaling complex. Clin Interv Aging. 2019;14:1579-87.
8. Dryn D, Melnyk M, Kizub I, Hu H, Soloviev A, Zholos A. The role of trpv4 cation channels in the regulation of phenylephrine-induced contraction of rat pulmonary artery. Fiziol Zh. 2016 ;62:79-86.
9. Chen K, Pittman RN, Popel AS. Nitric oxide in the vasculature: Where does it come from and where does it go? A quantitative perspective. Antioxid Redox Signal. 2008; 10:1185.
10. Zhao Y, Vanhoutte PM, Leung SWS. Vascular nitric oxide: Beyond eNOS. J Pharmacol Sci. 2015;129:83-94.
11. Argunhan F, Brain SD. The vascular-dependent and -independent actions of calcitonin gene-related peptide in cardiovascular disease. Front Physiol. 2022;13.
12. Aalkjaer C, Nilsson H, De Mey JGR. Sympathetic and sensory-motor nerves in peripheral small arteries. Physiol Rev. 2021;101:495-544.
13. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria. 2021.
14. Wei R, Lunn SE, Tam R, Gust SL, Classen B, Kerr PM, et al. Vasoconstrictor stimulus determines the functional contribution of myoendothelial feedback to mesenteric arterial tone. J Physiol. 2018;596:1181-97.
15. Ellinsworth DC, Earley S, Murphy TV, Sandow SL. Endothelial control of vasodilation: Integration of myoendothelial microdomain signalling and modulation by epoxyeicosatrienoic acids. Pflüg Arch. 2014;466: 389-405.
16. Wen JY, Zhang J, Chen S, Chen Y, Zhang Y, Ma ZY, Zhang F, Xie WM, Fan YF, Duan JS, Chen ZW. Endothelium-derived hydrogen sulfide acts as a hyperpolarizing factor and exerts neuroprotective effects via activation of large-conductance Ca^{2+}-activated K^+ channels. Br J Pharmacol. 2021 Oct;178(20):4155-75.
17. Strutynskyi RB, Strutynska NA, Piven OO, Mys LA, Goshovska YV, Fedichkina RA, Okhai IY, Strutynskyi VR, Dosenko VE, Dobrzyn P, Sagach VF. Upre-

gulation of ATP-sensitive potassium channels as the potential mechanism of cardioprotection and vasorelaxation under the action of pyridoxal-5-phosphate in old rats. J Cardiovascul Pharmacol Ther. 2023 Jan-Dec;28:10742484231213175.
18. Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH. K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature. 1998;396:269-72.
19. Hristovska AM, Rasmussen LE, Hansen PBL, Nielsen SS, Nüsing RM, Narumiya S, et al. Prostaglandin E2 induces vascular relaxation by E-prostanoid 4 receptor-mediated activation of endothelial nitric oxide synthase. Hypertension. 2007; 50:525-30.
20. Wilkinson JD, Kendall DA, Ralevic V. Δ9-Tetrahydrocannabinol inhibits electrically-evoked CGRP release and capsaicin-sensitive sensory neurogenic vasodilatation in the rat mesenteric arterial bed. Br J Pharmacol. 2007;152:709-16.
21. Werner M, Ledoux J. K^+ channels in biological processes: vascular K^+ channels in the regulation of blood pressure. J Rec Ligand Channel Res. 2014;7:51.
22. Filosa JA, Yao X, Rath G. TRPV4 and the regulation of vascular tone. J Cardiovascul Pharmacol. 2013;61:113-9.
23. Nagappan AS, Varghese J, Pranesh GT, Jeyaseelan V, Jacob M. Indomethacin inhibits activation of endothelial nitric oxide synthase in the rat kidney: possible role of this effect in the pathogenesis of indomethacin-induced renal damage. Chem Biol Interact. 2014; 221:77-87.
24. De Angelis A, Rinaldi B, Capuano A, Rossi F, Filippelli A. Indomethacin potentiates acetylcholine-induced vasodilation by increasing free radical production. Br J Pharmacol. 2004;142:1233.

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