THE EFFECTS OF GLYCERL TRINITRATE AND ADENOSINE 5-TRIPHOSPHATE ON ACTIVATION OF POTASSIUM CHANNEL-MEDIATED VASORELAXATION IN FEMALE RATS AORTIC SMOOTH MUSCLE.

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ABSTRACT:

Nitric oxide (NO) is produced from virtually all cell types composing the cardiovascular tissue and regulates vascular function through fine regulation of excitation–contraction coupling. Endogenous metabolites play a major role in coronary autoregulation. Therefore, the aim of the present study is to investigate the contribution of Glyceryl trinitrate (GTN) and Adenosine 5-triphosphate (ATP) mediated relaxation in rat aortic smooth muscle in intact and endothelium denuded endothelium rings precontracted with Phenylephrine (PE). The thoracic aorta was isolated, cut into rings, and mounted in organ-bath chambers and isometric tension was recorded using PowerLab Data Acquisition System (Model ML 870). The results showed that GTN as NO donor produced dose-dependent relaxation in intact aortic rings precontracted with PE (1 µM) that disinhibited in the presence of Glibenclamide (GLIB), while GLIB attenuate the response induced by ATP in intact aortic rings. L-nitroarginine methylester (L-NAME) an antagonist for nitric oxide synthases (NOS), not abolish the response induced by GTN (*E*max 55.28% ± 0.18). Caffeine, ATP receptors antagonist, were partially inhibit the relaxation induced by ATP (vasodilation rate decreased by about 20.57 %). In endothelium denuded aortic rings, vasorelaxation induced by ATP were significantly attenuated , while GTN significantly increased relaxation by removing endothelium. These results suggested that (1) ATP-dependent potassium channel did not involve in GTN inducing vasorelaxation while K_{ATP} and A_{2B} receptors have a role in ATP mediated vasorelation (2) ATP partially dependent on endothelium in contrast to NO donors that independent to endothelium.

Keywords: *Nitric oxide, Glyceryl trinitrate, Adenosine triphosphat, Potassium channels, Aorta.*

INTRODUCTION

Nitroglycerin (glyceryl trinitrate, GTN) is widely used for the treatment of angina pectoris. It is believed that the beneficial therapeutic effect of GTN is due to selective vasodilation of coronary arteries and venous capacitance vessels with minimal effect on arteriolar tone (Kleschyov *et al*., 2003). The antianginal drug GTN causes vasodilation through NO-mediated activation of vascular sGC (Matteo *et al*., 2008).

Studies have shown that NO can activate soluble guanylyl cyclase (sGC) and increase the level of cyclic Guanosine monophosphate (cGMP) in vascular tissue. This pathway of cGMP/ Protien kinase G (PKG) plays a great role in endothelium vasorelaxation (Maneesai *et al*., 2016) and (Bailey, Feelisch, Horowitz, Frenneaux, & Madhani, 2014). PKG, elicits relaxation in vascular smooth muscle cells VSMCs through a numerous of signaling pathways, leading to decreased intracellular calcium ion concentration $[Ca^{2+}]_i$ and desensitization of the contractile apparatus to Ca^{2+}. However, evidences exist for PKG-dependent activation of large-conductance Ca^{2+}-activated K^+ (K_{Ca}) channels and associated membrane hyperpolarization, inhibition of L-type voltage-gated Ca2+ channels, stimulation of Ca^{2+}-ATPases in both the plasma membrane and sarcoplasmic reticulum and inhibition of inositol trisphosphate receptors (Carvajal, Germain, Huidobro-Toro, & Weiner, 2000)and (Gewaltig & Kojda, 2002).

Adenosine 5-triphosphate (ATP) is an important nucleotide with various functions including diverse effects on the cardiovascular system (Crecelius et al., 2011). Smooth muscle cells express ligand-gated P2X receptors (P2XR) and G-protein-coupled P2Y receptors (P2YR), there is an emerging role of purinergic receptors as therapeutic targets in hypertension (Neshat *et al*., 2009). In vitro studies in a variety of tissues have demonstrated that ATP-mediated vasodilation is endothelium dependent and occurs through the activation of endothelial G-protein-coupled P2Y receptors(Crecelius, *et al*., 2011). Many of the physiological effects of neuronally released ATP in smooth muscle are influenced by the relaxant actions of P2YR

which are largely coupled to $G\alpha_q$ proteins subunits and thus to the activation of phospholipase C (PLC) Indeed, the direct inhibitory response to ATP on smooth muscle has been proposed to involve PLC mediated phosphoinositide hydrolysis and the subsequent ATP dependent production of Ins(1,4,5)P3 to evoke local Ca2+ release near the plasma membrane via Ins(1,4,5)P3Rs. The [Ca2+]c rise, it is proposed, may activate Ca2+-activated K+ (KCa) channels to hyperpolarize the plasma membrane and decrease bulk average [Ca2+] (MacMillan, Kennedy, & McCarron, 2012).

The current study was designed to evaluate the contribution of potassium channel to GTN and ATP mediated relaxation in precontracted descending thoracic aorta. Furthermore, to find out the role of endothelium on NO and endogenous metabolites mediated aortic relaxation.

MATERIALS AND METHODS

TISSUE PREPARATION

Female Albino rats (*Rattus norvegicus*) (200-270 g) were used for this study. The animals were kept under standard laboratory conditions. The animal experimental procedures conformed to the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH) in the United States and was approved by the Animal Research Committee of Zakho University. The animal were injected intraperitoneally with heparin (2000 units/ 200 g) for several minutes to avoid blood clotting and damaging of aortic endothelium. After anesthetization, the descending thoracic aortae was carefully isolated and transferred immediately to Kreb's bicarbonate, which compose of followings (in mM): NaCl, 118; KCl, 4.7; KH2PO4, 1.2; MgSO4, 1.2; NaHCO3, 15.0; Glucose, 5.5; CaCl2, 2.5. The aorta was cleaned of periadventitial tissue in cold krebs solution and cut transversally into ring segments (each of 3 mm in length) and (Shekha & Al-Habib, 2013).The rings were placed in a 10-ml organ chamber containing Krebs solution maintained at 37^{o}C. Two stainless-steel wires were passed through the lumen of each ring. One stirrup was connected to an isometric force transducer (Model FORT100) to measure tension in the vessels and connected to a PowerLab data acquisition system (Model ML845, AD Instruments, Australia). A computer running chart software (version 7.0) was used for the measurement of isometric tension. The rings were stretched until they exerted an optimal basal tension of 2 g, and then were allowed to equilibrate for 60 minutes with the bath fluid being changed every 15–20 minutes (Deveci, 2006). The solution was bubbled with a mixture of 95% O2/5% CO2. In experiments with denuded endothelium, the endothelium was mechanically removed by gently rubbing the lumen of the vessel with a syringe needle covered by a piece of cotton. Endothelial integrity was assessed qualitatively by the degree of relaxation caused by acetylcholine (10 μM) in the presence of contractile tone induced by phenylephrine. In the studies of endothelium-intact vessels, if relaxation with acetylcholine was not 80% or greater, the ring was discarded. In the studies of endothelium-denuded vessels, the rings were discarded if there was any degree of relaxation.

Evaluation of the Mechanisms Underlying the Relaxant Effect Induced by GTN and ATP

Endothelium-intact and endothelium-denuded tissues were precontracted with phenylephrine, used in the concentration of (1μM). After the rings had reached a stable and sustainable contraction, a (1×10^{-7} to 3×10^{-4} M) was added cumulatively to the organ bath.

To test the effect of the role of K_{ATP} channels in the development of relaxation, the aortic rings were preincubated with the 10 μM GLIB and Caffeine (300μM). To test the effect of blocking NO synthases in the presence of GTN, the aortic rings were preincubated with L-NAME (3×10^{-4}M). All drugs were present for 30minutes before precontraction with PE and experimental procedures. At these concentrations the drugs did not change the basal tonus of the aortic rings.

Statistical Analyses

Results are expressed as means ± SEM. The Log median effective concentrations (IC_{50}) are given as geometric mean with 95% confidence intervals (CI). The GTN and ATP induced relaxation is expressed as percentage change from the phenylephrine-contracted levels. Agonist Concentration–Response curves were fitted using a nonlinear interactive fitting program (Graph Pad Prism 5.0 (Graph Pad Software, USA). For comparison between means of two groups two ways ANOVA, Bonferroni test was used. P-values less than 0.05 were considered as statistically significant.

RESULTS

Effect of GLIB on GTN and ATP Inducing Vasodilation of Isolated Rats Aorta

Contraction to (1μM) of PE was reduced in vessel preincubated with GLIB and treated cumulatively with different concentrations of GTN in which the response was reduced by (2.42 %) in comparison with control (E_{max} 57.63% ±3.7 and 60.05% ± 0.81) (Log IC50 -4.978 and -5.137) respectively. Therefore and in accordance with our data, a specific K_{ATP} blocker GLIB (10 μM) disinhibited the relaxation produced by GTN. While, on the other hand, the data of the current study showed that GLIB abolished the relaxation potentiated by ATP comparison with control (The relaxation response was decreased from 47.01± 2.19% control to 14.93±5.67% GLIB) (Log IC50 -5.426 control VS -5.244 GLIB) .

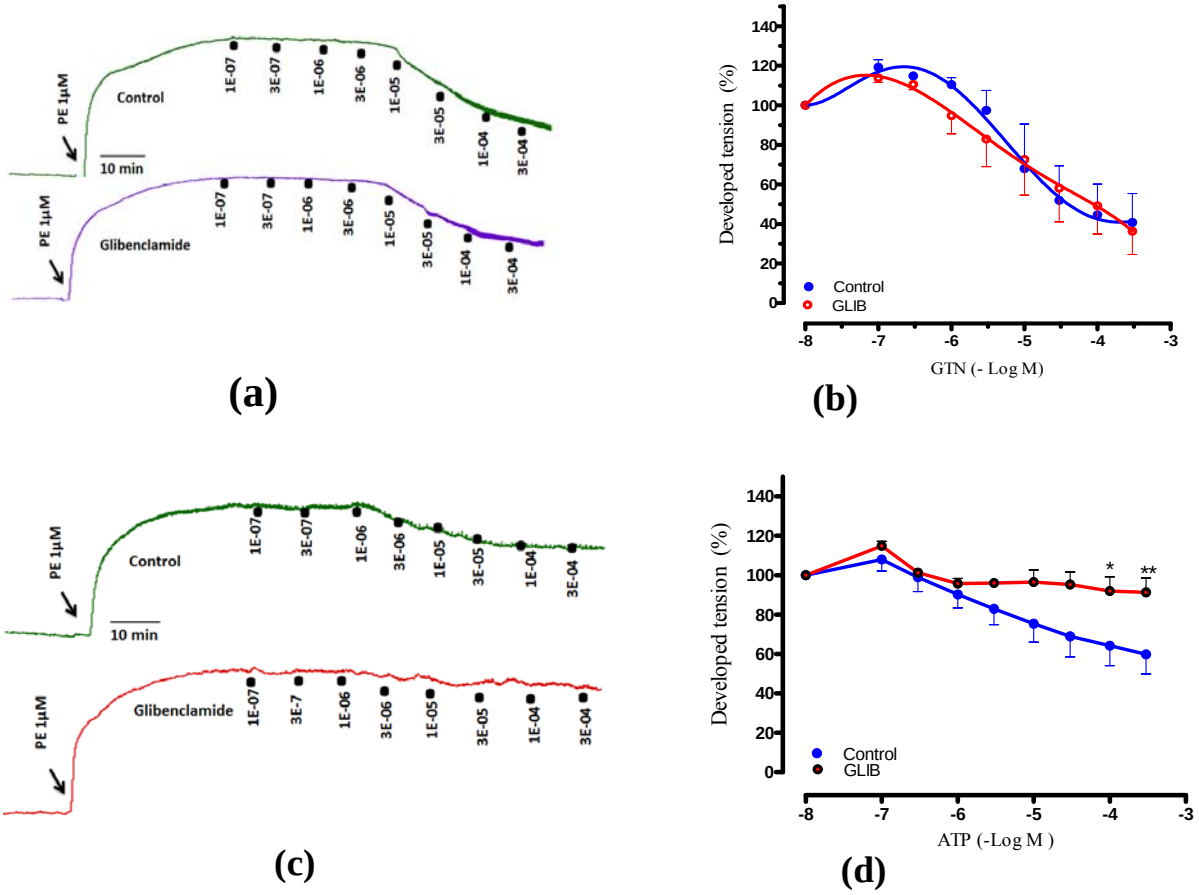
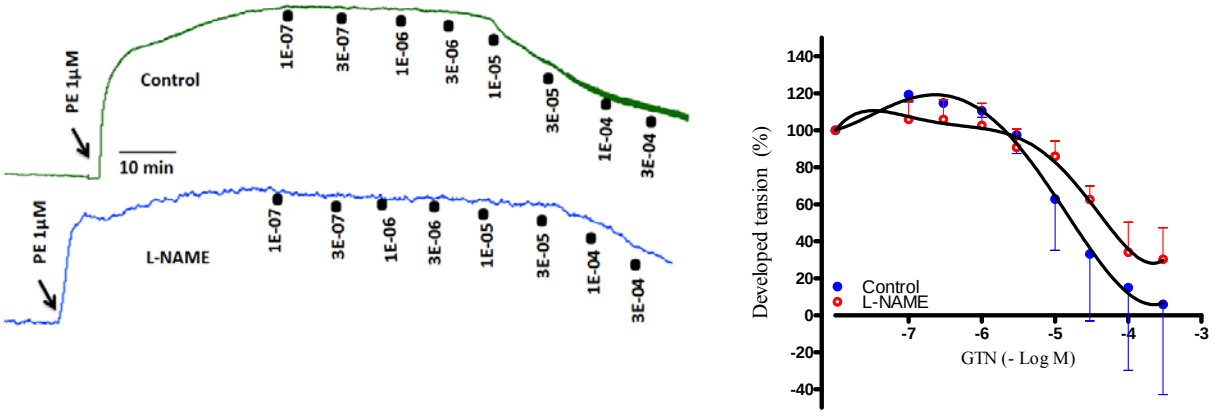


Figure1. Concentration-response effects of GTN and ATP on PE (1 μM)-induced vasoconstriction. (a) Typical chart view trace and (b) Dose-response curve showing comparative vasorelaxation effects of GTN on PE-induced vasoconstriction (control) and GLIB preincubated aortic rings, (c) Typical chart view trace and (d) Dose-response curve showing comparative vasorelaxation effects of ATP on PE-induced vasoconstriction (control) and GLIB preincubated aortic rings. In chart trace ● indicates addition of GTN (M) in cumulative manner and for each dose 3 min. (*P < 0.05; compared to control; Two-way ANOVA, Bonferroni posttest).

Effect of L-NAME on GTN Inducing Vasodilation of Rats Isolated Aorta

To investigate whether L-NAME as NOS antagonist have role in increasing vasorelaxation induced by GTN concentrations, (3×10^{-4}M) L-NAME in intact endothelium aortic rings were used. As in previous experiments different concentrations of GTN were added to the aortic rings precontracted with PE (1μM) and preincubated with L-NAME in organ bath experiments. Vasodilation that produced in response to GTN in presence of L-NAME decreased slightly but not abolished (E_{max} 55.28±6.72% L-NAME VS 60.05± 0.81% control) and (Log IC50 -4.571 L-NAME VS -4.978 control).

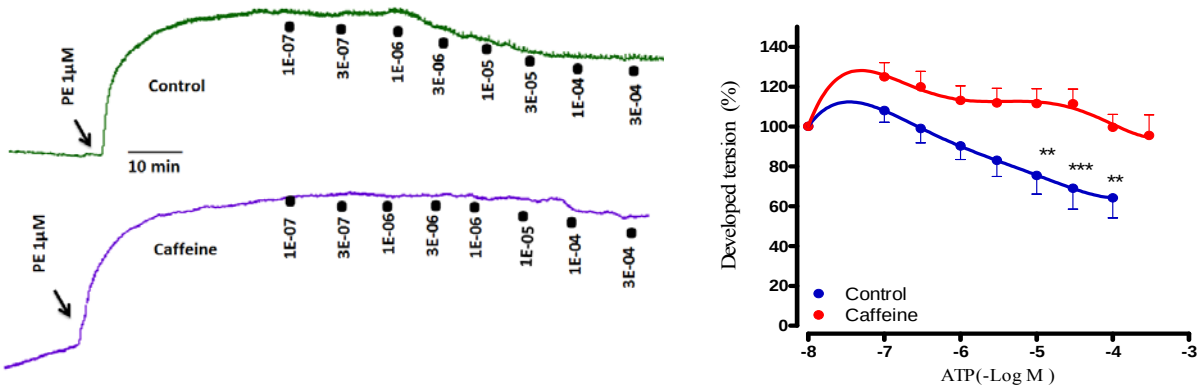


(a) **(b)**

Figure2. Concentration-response effects of GTN on PE (1 µM) induced vasoconstriction. (a) Typical chart view trace and (b) Dose-response curve showing comparative vasorelaxation effects of GTN on PE-induced vasoconstriction (control) and L-NAME preincubated aortic rings. In chart trace ● Indicates addition of GTN (M) in cumulative manner for each dose 3 min. (*P < 0.05; compared to control; Two-way ANOVA, Bonferroni post test).

Effect of Caffeine on GTN Inducing Vasodilation of Rats Isolated Aorta

To test the role of Purinergic receptors in mechanism of vasodilation induced by agonist ATP Caffeine were used. Caffeine in accordance to the data of present work partially inhibit vasorelaxation induced by ATP (E_{max} 47.01± 2.19% control VS 26.44±1.51% caffeine) (Log IC50 -5.426 control VS -4.192 caffeine).



(a) **(b)**

Figure3. Concentration-response effects of ATP on PE (1 µM)-induced vasoconstriction. (a) Typical chart view trace and (b) Dose-response curve showing comparative vasorelaxation effects of ATP on PE-induced vasoconstriction (control) and Caffeine preincubated aortic rings. In chart trace ● indicates addition of ATP (M) in cumulative manner and for each dose 3 min. (*P < 0.05; compared to control; Two-way ANOVA, Bonferroni post test).

Role of GTN and ATP in Inducing Relaxation in Endothelium Denuded Rats Aortic Rings

To assess the role of endothelium in producing the vasorelaxation, endothelium denuded aortic vessels was used and treated with different concentrations of GTN and ATP. The conserved results proved that the relaxation produced by GTN is not affected by removing endothelium, the relaxation rate were about (55.01%) (Log IC50 -4.978 control VS -4.99 denuded endothelium). While, on the other hand, relaxation rate in endothelium denuded rings decrease when treated with ATP (E_{max} 47.01± 2.19% control to 16.78 ± 0.546 % endothelium denuded) (Log IC50 -5.426 control VS -5.371 endothelium denuded).

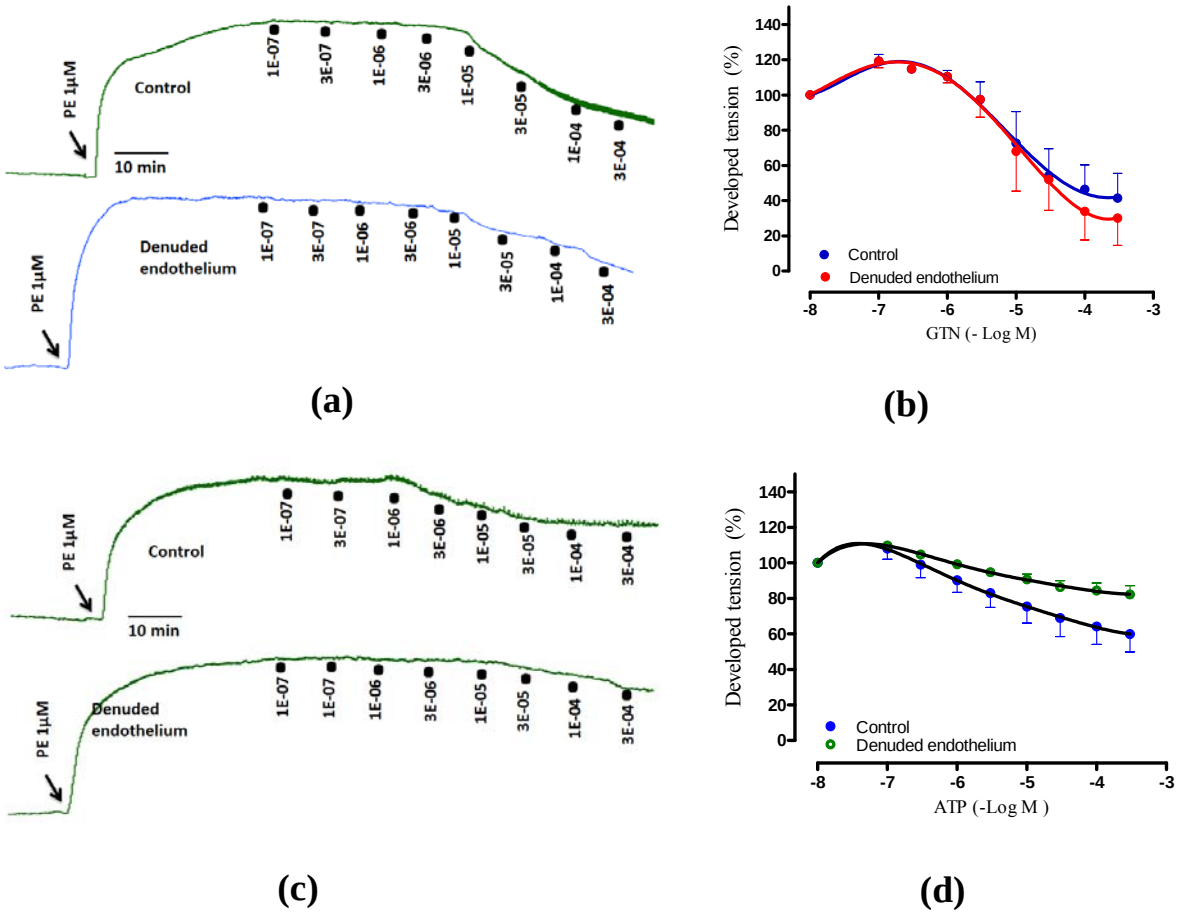


Figure4. Concentration-response effects of GTN and ATP on PE (1 µM)-induced vasoconstriction. (a) Typical chart view trace and (b) Dose-response curve showing comparative vasorelaxation effects of GTN on PE-induced vasoconstriction (control) and endothelium denuded aortic rings, (c) Typical chart view trace and (d) Dose-response curve showing comparative vasorelaxation effects of ATP on PE-induced vasoconstriction (control) and GLIB preincubated aortic rings. In chart trace ● indicates addition of GTN (M) in cumulative manner and for each dose 3 min. (*P < 0.05; compared to control; Two-way ANOVA, Bonferroni post test).

DISCUSSION

In the present study inhibiting of vasoconstriction induced by PE in aortic smooth muscle by GTN in the presence of GLIB indicated that the hyperpolarization produced by this NO donor did not involve the activation of ATP-activated potassium channel and K_{ATP} channels may not be the only signaling mechanism responsible for the vasorelaxation. On the other hand Wellman and coworker suggested that, NO can relax smooth muscles by activating of K_{Ca} channel (Wellman & Nelson, 2003).

To test either vasorelaxation that produced by GTN will decrease or not in the presence of NOS blocker, L-NAME was used. The current study showed the NOS inhibitor effect of L-NAME did not attenuate or completely abolished GTN -induced vasodilation in rats aortic smooth muscle (Salihi & Al-Habib, 2013). However the E_{Max} in presence of L-NAME slightly decreased as compared to control, suggesting the possible nonenzymatic release of NO from GTN because GTN activated sGC only in the presence of low molecular weight thiols, and NO was found to be a direct activator of sGC, it was proposed that this free radical might mediate the bioactivity of GTN.

Inhibition of endothelial K_{ATP} channels by GLIB abolished ATP-induced vasodilation in aortic smooth muscle cells, and the inhibiting of vasodilation by GLIB was not similar to that produced by endothelial removal. This response may not be due to the contribution of not only endothelium K_{ATP} but also smooth muscle in vasodilation induced by adenosine receptor when activated by ATP after ATP degraded to adenosine (Ho, Low, & Rose'Meyer, 2016).

In the present study, we found that the dilation elicited by ATP was not inhibited but attenuated when preincubated with caffeine by which the maximum effect of dilation was reduced by about 20±7 %, these results indicate that ATP has role in vasorelaxation. On the other hand, in the presence of caffeine, ATP at high concentration significantly increase response which may be due to different caffeine actions in endothelium and SMC such as reduction of cytoplasmic Ca2+ in VSMCs through cyclic adenosine monophosphate (cAMP) and the increase of Ca2+ in the endothelial cell, favoring the synthesis of NO (Al-Habib & Muhammmad, 2014).

The role of the endothelium in dilation of aortic rings to GTN and ATP is controversial. So we examined the role of endothelium-derived NO in endothelium denude rings. According to data from this study GTN independent to endothelium. Our present studies using isolated aorta demonstrated that the vasodilation to ATP is partially dependent on the endothelium, given that the vasodilator response by ATP achieved by more than one channel in SMC.

REFERENCES

Al-Habib, O. A. M., & Muhammmad, C. M. (2014). Sodium Nitroprussid and Adenosine-Activated Potassium Channel in Aortic Smooth Muscle Isolated From Female Rats. *Journal of University of Zakho, 2(A)*, 1-7.

Bailey, J. C., Feelisch, M., Horowitz, J. D., Frenneaux, M. P., & Madhani, M. (2014). Pharmacology and therapeutic role of inorganic nitrite and nitrate in vasodilatation. *Pharmacol Ther, 144*(3), 303-320.

Carvajal, J. A., Germain, A. M., Huidobro-Toro, J. P., & Weiner, C. P. (2000). Molecular mechanism of cGMP-mediated smooth muscle relaxation. *J Cell Physiol, 184*(3), 409-420.

Crecelius, A. R., Kirby, B. S., Richards, J. C., Garcia, L. J., Voyles, W. F., Larson, D. G., . . . Dinenno, F. A. (2011). Mechanisms of ATP-mediated vasodilation in humans: modest role for nitric oxide and vasodilating prostaglandins. *Am J Physiol Heart Circ Physiol, 301*(4),

Deveci, E. (2006). Ultrastructural effects of lead acetate on brain of rats. *Toxicol Ind Health, 22*(10), 419-422.

Gewaltig, M. T., & Kojda, G. (2002). Vasoprotection by nitric oxide: mechanisms and therapeutic potential. *Cardiovasc Res, 55*(2), 250-260.

Ho, M. F., Low, L. M., & Rose'Meyer, R. B. (2016). Pharmacology of the Adenosine A3 Receptor in the Vasculature and Essential Hypertension. *PLoS One, 11*(2), e0150021. doi: 10.1371/journal.pone.0150021 PONE-D-15-47379 [pii]

Kleschyov, A. L., Oelze, M., Daiber, A., Huang, Y., Mollnau, H., Schulz, E., . . . Munzel, T. (2003). Does nitric oxide mediate the vasodilator activity of nitroglycerin? *Circ Res, 93*(9), e104-112.

MacMillan, D., Kennedy, C., & McCarron, J. G. (2012). ATP inhibits Ins(1,4,5)P3-evoked Ca2+ release in smooth muscle via P2Y1 receptors. *J Cell Sci, 125*(Pt 21), 5151-5158.

Maneesai, P., Prasarttong, P., Bunbupha, S., Kukongviriyapan, U., Kukongviriyapan, V., Tangsucharit, P., . . . Pakdeechote, P. (2016). Synergistic Antihypertensive Effect of Carthamus tinctorius L. Extract and Captopril in L-NAME-

Induced Hypertensive Rats via Restoration of eNOS and AT(1)R Expression. *Nutrients, 8*(3), 122.

Matteo, B., Karl, G., Alexander, K., Michael, R., Doris, K., Walter, G., Bernd, M. (2008). Bioactivation of Nitroglycerin by Purified Mitochondrial and Cytosolic Aldehyde Dehydrogenases. *J Biol Chem. , 283*((26)), 17873–17880.

Neshat, S., deVries, M., Barajas-Espinosa, A. R., Skeith, L., Chisholm, S. P., & Lomax, A. E. (2009). Loss of purinergic vascular regulation in the colon during colitis is associated with upregulation of CD39. *Am J Physiol Gastrointest Liver Physiol, 296*(2), G399-405.

Salihi, A. B. Q., & Al-Habib, O. A. M. (2013). The Role of Endothelium and Endothelium-Derived Relaxation Factors in Nitric Oxide-Induced Aortic Relaxation. Journal of University of Zakho, 1(A): 95-100

Shekha, M. S., & Al-Habib, O. A. M. (2013). Relaxant Effects of Rutin on Rat Smooth Muscles. *Journal of University of Zakho, 1,(A)*(2), 139-144.

Wellman, G. C., & Nelson, M. T. (2003). Signaling between SR and plasmalemma in smooth muscle: sparks and the activation of Ca2+-sensitive ion channels. *Cell Calcium, 34*(3), 211-229. doi: S0143416003001246 [pii]

پوختە

نایتریك ئوكساید (NO) دهێتە بەرهەم هێنان ل زوربەی خانێن دل و لوولەی و كاردكەن بۆ رێكخستنا كارێ لوولەی ب رێكخستنەكا هویر بۆ پروسێسا هاندان و كرژبونێ.ATP كو رولەكێ سەرەكی یێ هەی ل رێك و پێك كرنا شاخوێنبەری. ژ بەر هندێ ئارمانجا ڤێ ڤەكولینێ دیاركرنا بەشدارکرنا گلیسیرایل تریناینریت GTN و ئەدینوسین تریفوسفەیت ATP ل خاوكرنا ماسولكێن لوس ل شاخوێنبەری ب خانێن داپوشەری و بێ داپوشەرێ هاتینە جوداكرن ژ چوردێن سپی كو بەری هنگی هاتینە كرژكردن ب ڤینایلفرین.

شاخوێنبەری ٍسینك هاتینە چوداكرن ژ چوردی و پارچەكریە بۆ پارچێن بازنەیی و هێلایە دناف (organ-bath chambers) و ژمارتنا شداندێ هاتیە تومارکردن بكارئینانا Power Labو Acquisition System Model ML 870 Data)

و لدیف ئەنجامێن ڤێ ڤەكولینێ ، GTN وەك دونەرێ NO رابویە ب خاوكرنا پارچێن شادەماری ب چركردنێت ئێك ل دیف ئێك, ئەوا كو نە هاتیە ژنافبرن ب كارئینانا GLIB د ژلایەكێ دیفە GLIB خاوبونا ماسولكا ب ژەمێن ژنافبرنو كێمكر كافایین وەك گرتگرێ وەرگرێت ئەدینوسینی خاوبونا ماسولكا ب ژەمێن كێمكر ب شێوەیەكێ بەشەیی ,كێمكرن نێزیكی هینده بو (20.57 %) ژ لایێ دیفە LNAM وەك رێگرێ ئینزیمێ نەشیا دەرئەنجامی كێمكە(*E*max 55.28% ± 0.18) . لابرنا داپوشەری ژی بروسێسا خاوبونێ كێم نەبو بەروڤاژی بروسێسا خاوبونێ بلندكر ژ لایێ ژەمێن ژبەلێ پاژلایێدیفە لابرنا داپوشەری بروسێسا خاوبونێ كێم بو .

الملخص

أكسيد النيتريك (NO) ينتج من جميع أنواع الأوعية الدموية القلب وينظم وظيفة الأوعية الدموية من خلال التنظيم الدقيق للاقتران الإثارةو تقلص. الأيض الذاتية تلعب دورا رئيسيا في تنظيم ذاتي التاجي. لذلك، كان الهدف من هذه الدراسة إلى فحص مدى مساهمة (GTN) وأدينوسين ثلاثي الفوسفات 5 (ATP) الاسترخاء توسط في الفئران الأبهر العضلات الملساء في حلقات البطانة سليمة والجرداء precontracted مع (PE) Phenylepherineالشريان الأورطي الصدري تم عزل ، مقطعة إلى حلقات، تم عزل الشريان ابهر ، وقطع إلى حلقات، وربط في الجهاز organ bath. تم تسجيل شدة العضلة باستخدام Data Acquisition System (Model ML 870 powerLab).

وفقا لنتائج هذه الدراسة، انخفضت استجابة الاسترخاء الناجم عن Ado في حلقات الأبهر المحضنة مع Glybenclamide(GLIB). L-nitroarginine methylester (L-NAME)، لم تلغ الارخاء الناجم عن GTN(*E*max 55.28% ± 0.18)، في ناحية أخرى GLIB ادت ادت الى انخيفاض كبر في الارخاء الناجم عن ATP بطريقة تعتمد على الجرعة في حلقات سليمة البطان L-nitroarginine methylester (L-NAME). وهو خصم لNO synthesase، لا يلغي استجابة الناجمة عن) GTN ايماكس 55.28٪ ± 0.18). الكافيين، ATPمستقبلات خصم، وتمنع جزئيا الاسترخاء الناجم عن) ATP معدل توسع الأوعية انخفضت بنحو (20.57٪). في الجرداء البطانة حلقات الأبهري، وارتخاء وعائي الناجمة عن ATP مخففة إلى حد كبير، في حين GTN زيادة كبيرة الاسترخاء عن طريق إزالة البطانة. وتشير هذه النتائج إلى أن (1) لم قناة البوتاسيوم ATP التي تعتمد لا تنطوي في GTN إحداث ارتخاء وعائي في حين KATP و A2Bمستقبلات لها دور في ATP vasorelation بوساطة. (2 ATP (يعتمد جزئيا على البطانة على النقيض من NO الجهات المانحة التي مستقلة لالبطانة.