SODIUM NITROPRUSSID AND ADENOSINE-ACTIVATED POTASSIUM CHANNEL IN AORTIC SMOOTH MUSCLE ISOLATED FROM FEMALE RATS

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Abstract:

Sodium Nitroprusside (SNP) and Adenosine (Ado) are potent drugs used in the treatment of cardiovascular diseases. Nitric oxid (NO) is produced from virtually all cell types composing the cardiovascular and regulates vascular function through fine regulation of excitation–contraction coupling. Adinosine endogenous metabolites play a major role in coronary autoregulation. Therefore, the aim of the present study was to investigate the contribution of NO and Ado mediated relaxation in rat aortic smooth muscle in intact and denuded endothelium rings precontracted with phenylepherine (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). According to the results of the current study, incubation of aortic rings with Glybenclamide (GLIB) decreased the relaxation response induced by Ado (the vasodilation value rate decrease from 41.07±6.7 control to 18.54±4.6) in intact aortic rings. L-nitroarginine methylester (L-NAME), not abolished the response induced by SNP, whereas Nifedipine significantly enhanced the response induced by SNP in a dose-dependent manner in intact endothelium rings. The relaxation to Ado in intact aortic rings was slightly decreased (6.88± 1.01), but not abolished completely after incubation with Caffeine (Ado receptors antagonist). On the other hand, removing endothelium did not attenuated the vasorelaxation induced by SNP and increased relaxation response. While, vasorelaxation of Ado in aortic rings were partially attenuated by removing endothelium. These results suggested that (1) ATP-dependent potassium channel (K_{ATP}) did not involve in SNP inducing vasorelaxation, while have a role in Ado mediated vasorelation. (2) Vasorelaxation effect of NO is endothelium independent, while, Ado relaxation effect is endothelium dependent.

Keywords: *Nitric oxide,Adenosine, Potassium channels, Aorta.*

INTRODUCTION

Nitric oxide (NO) and adenosine (Ado) mediates multiple physiological and pathophysiological processes in cardiovascular system (Ignarro *et al*., 2002). NO is known as a primary determinant of blood vessel tone and thrombogenicity, however the modulatory effects of NO on contractile function are undoubtedly complex (Massion *et al*., 2003). NO donor causes an increase in cyclic Guanosine monophosphate (cGMP) concentration, cGMP in turn stimulates Protein Kinas G (PKG) (Pfitzer 2001; Lincoln *et al*., 2001). PKG, elicits relaxation in vascular smooth muscle cells through a myriad of signaling pathways, leading to decreased intracellular calcium ion concentration $[Ca^{2+}]_i$ and desensitization of the contractile apparatus to Ca^{2+} (Carvajal *et al*., 2000). 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 Ca^{2+} channels, stimulation of Ca^{2+}-ATPases in both the plasma membrane and sarcoplasmic reticulum and inhibition of inositol trisphosphate receptors (Lincoln *et al*., 2001). Furthermore, NO can promote vascular relaxation through cGMP-independent mechanisms of smooth muscle relaxation by nitrosylation of cysteine thiol groups to post-translationally modify enzymatic activity (Resta, 2003). Lin *et al*., 2007 have been suggested that L-NAME, a competetive NO inhibitor, inhibit NO release.

Endogenous metabolites have been postulated to play a major role in coronary autoregulation (Makujina *et al*., 1994). The vasodilator action of Ado has generally been ascribed to stimulation of the A_2 Ado receptor subtype, probably acting by activation of adenylyl cyclase (AC) leading to elevation of cyclic adenosine monophosphate (cAMP) levels (Dart and Standen, 1993). However, it is unclear how A_{2A} receptor modulates the vascular response. In the guinea pig, A_{2A} Ado receptor is involved in coronary vessel relaxation, whereas A_{2B} receptor is present predominately in the aorta. Similarly, the vascular effects of adenosine in aorta and coronary vessel of rat have been reported to be mediated by both A_{2A} and A_{2B} receptors (Ponnoth *et al*., 2009).

The signal transduction pathway between Ado receptors and K_{ATP} channels in VSM is still

unknown. K_{ATP} channels in smooth muscle from mesenteric arteries and gallbladder can be activated through stimulation of cAMP-dependent protein kinase A (PKA) (Quayle *et al*., 1994).

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

MATERIALS AND METHODS

Animals

Female Albino rats (200-270 gm in weight) were used in the present study. Animals were housed in the animal house of Biology Dept., Faculty of Science, Zakho of University. Animals were kept at 22±2 °C and exposed to a regular diurnal cycles of 12-hours photoperid using an automated light-switching devise and had free access to water and food ad *libitum*.

Tissue preparation

The animals were injected intrapretoneally with heparin (2000 units/ 200 gm) and left for few minutes to avoid blood clotting and damaging of aortic endothelium. The Animals were anesthetized by placing them in a small cage and allowing them to inhale Diethyl ether (Deveci, 2006). Then, the descending thoracic aortae was carefully isolated and transferred immediately to Kreb's bicarbonate buffer solution with glucose and EDTA-to prevent the oxidation of unstable substances. The aorta was cleaned of periadventitial tissue and cut transversally into ring segments (each of 3 mm in length).

Measurement of vascular reactivity in isolated rat aorta

Each aortic ring was placed in a tissue bath filled with Kreb's buffer (37 ^{o}C), bubbled with carbogen (95% O_2 and 5% CO_2), and attached to a force transducer (Model FORT100) and connected to a PowerLab data acquisition system (Model ML845, ADInstruments, Australia). Computer running Chart software (version 7.0) was used for the measurement of isometric tension. Rings were allowed to equilibrate for 60–90 min at a resting tension of 2 g, before the addition of the blockers. The aortic segments were initially exposed to 60 mM K^+ to test their functional integrity. Later, the bath medium was changed several times until a resting tone was restored.

To test the role of K_{ATP} channels in the development of relaxation, the aortic rings were pre-incubated for 30 minutes with the 10 µmol/l Glibenclamide (K_{ATP} inhibitor) and Caffeine (3×10^{-4}mM). To test the effect of blocking NO synthetase in the presence of SNP, the aortic rings were preincubated with L-NAME. To investigate the effect of Nefedepine on SNP inducing vasorelaxation, the rings were pretreated with (10^{-5}mM) Nefedepine. Endothelial injury was induced by gentle rubbing of the intimal surface of the rings with a piece of PE 90 tubing and checked by the addition of Acetylcholine. Denuded endothelium rings were treated with SNP and Ado. Each ring was then contracted with PE (1×10^{-6} mM). Once a stable contraction was reached, cumulative concentration-response curves were obtained for SNP and Ado (1×10^{-7} to 3×10^{-4} mM).

Statistical analysis

All the data were expressed as means ± SEM. The median effective concentrations (IC_{50}) are given as geometric mean with 95% confidence intervals (CI). For comparison between means of two groups, two way ANOVA was used. P-values less than 0.05 were considered as statistically significant. All the graphs, calculation and statistical analyses were performed using GraphPad Prism software version 5 (GraphPad Software, USA).

RESULTS

Effect of GLIB on SNP and Ado Inducing Vasodilation

The involvement of K_{ATP} channel in inducing vasodilation to SNP and Ado was examined pharmacologically. A specific K_{ATP} channel blocker GLIB (1×10^{-5}M) was administrated to vessels to block the K_{ATP} channel activity. The results showed that the relaxation of the aorta to different concentrations of SNP pretreated with GLIB disinhibited and reduced vasoconstriction induced by PE in which the relaxation effect was reduced by 2.1±0.7.

The data of the current study showed the abolishing of the relaxation response potentiated by Ado in the presence of extracellular GLIB in PE (10^{-6}M) precontracted rats aorta and relaxation response was decreased from 41.07±6.7 control to 18.54±4.6 .

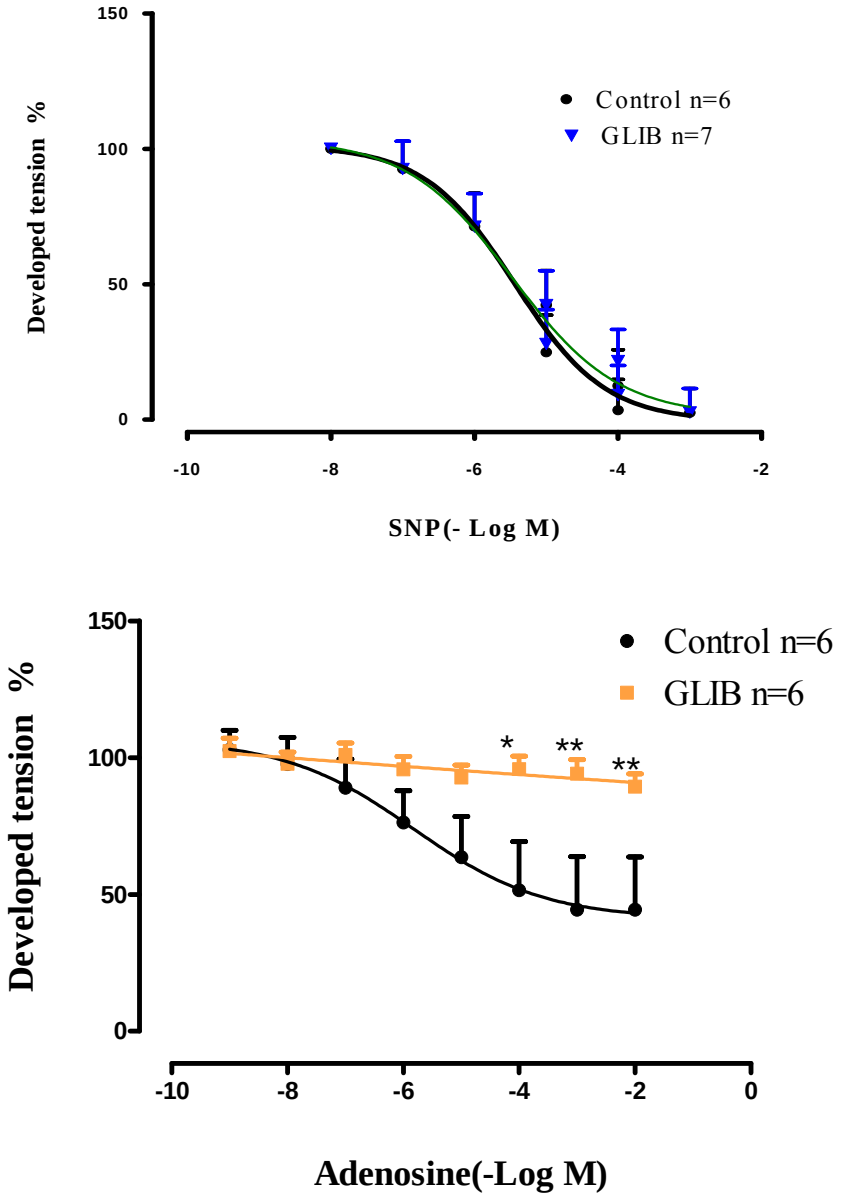

Fig 1. The effect of GLIB on the relaxation response induced by SNP and Ado in PE precontracted rat aortic rings. (A and B) Dose-response curve to SNP and Ado induced relaxation in control and in preincubated aortic rings with GLIB**.**

Effect of L-NAME on SNP Inducing Vasodilation

To investigate whether L-NAME as NOS antagonist have ability to abolish vasorelaxation induced in response to different SNP concentrations, we treat intact endothelium aortic rings with (3×10^{-4}M) L-NAME. As in previous experiments different concentrations of SNP were added to the aortic rings precontracted with PE (10^{-6}M) and preincubated with L-NAME in organ bath experiments. Vasodilation that produced in response to SNP in presence of L-NAME was decreased slightly but not abolished and vasodilation rate decreased by about 10.14 ±0.05.

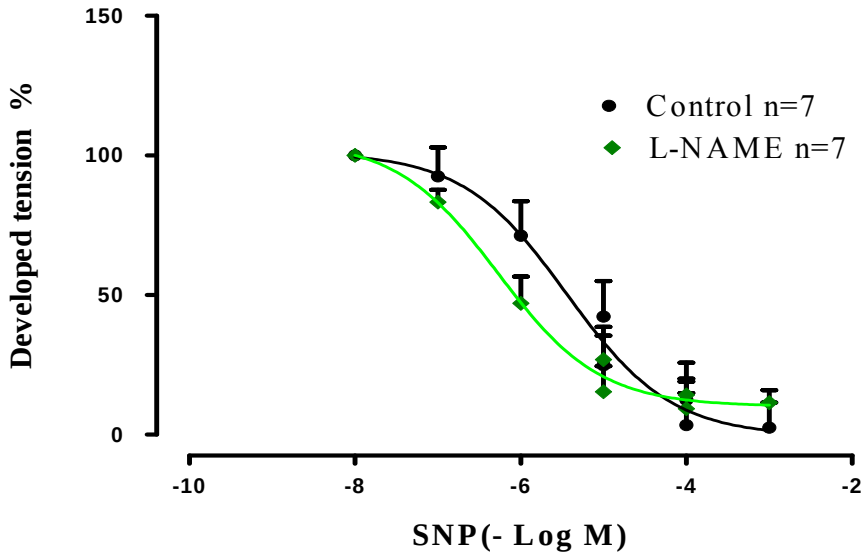

Fig 2. The effect of L-NAME on the relaxation reponse to SNP in PE precontracetd rat aortic rings. Dose-response curve to SNP and GTN induced relaxation in control and in preincubated rings with L-NAME.

Effect of Nifedipine on SNP Induced Relaxation

Nifedipine caused more rapid relaxation in aortic rings at low concentration of SNP (3×10^{-7}M), and the vasodilation produced was increased in comparatively by about 6.43±1.9 with Log IC50 -5.770.

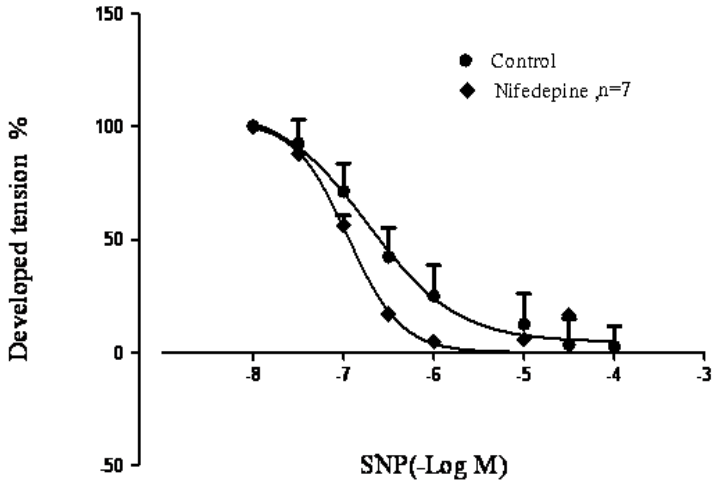

Fig 3. The effects of Nefedipene on the relaxant response to SNP in PE precontracted rat aortic rings. Dose response curve to SNP induced relaxation in control and in preincubated aortic rings with Nefedepine.

Role of Endothelium in SNP and Ado Inducing Vasorelaxation

To assess the role of endothelium in producing the vasorelaxation, the endothelium was initially removed to eliminate its contribution to vasodilation. Denuded endothelium aortic vessels were treated with different concentrations of SNP. The results showed that the relaxation produced by SNP is not affected by removing endothelium and the relaxation rate was reduced only by 3.3±1.1 with a Log IC50 -5.683. On the other hand, results showed that the disruption of endothelium attenuated Ado - induced vasodilation, and the relaxation was comparatively decreased from 47.37% in the control to 10.37%, with Log IC50 -5.105, -5.245 respectively, and the response of Ado relaxation at 3×10^{-3} mM was significant ($P<0.05$).

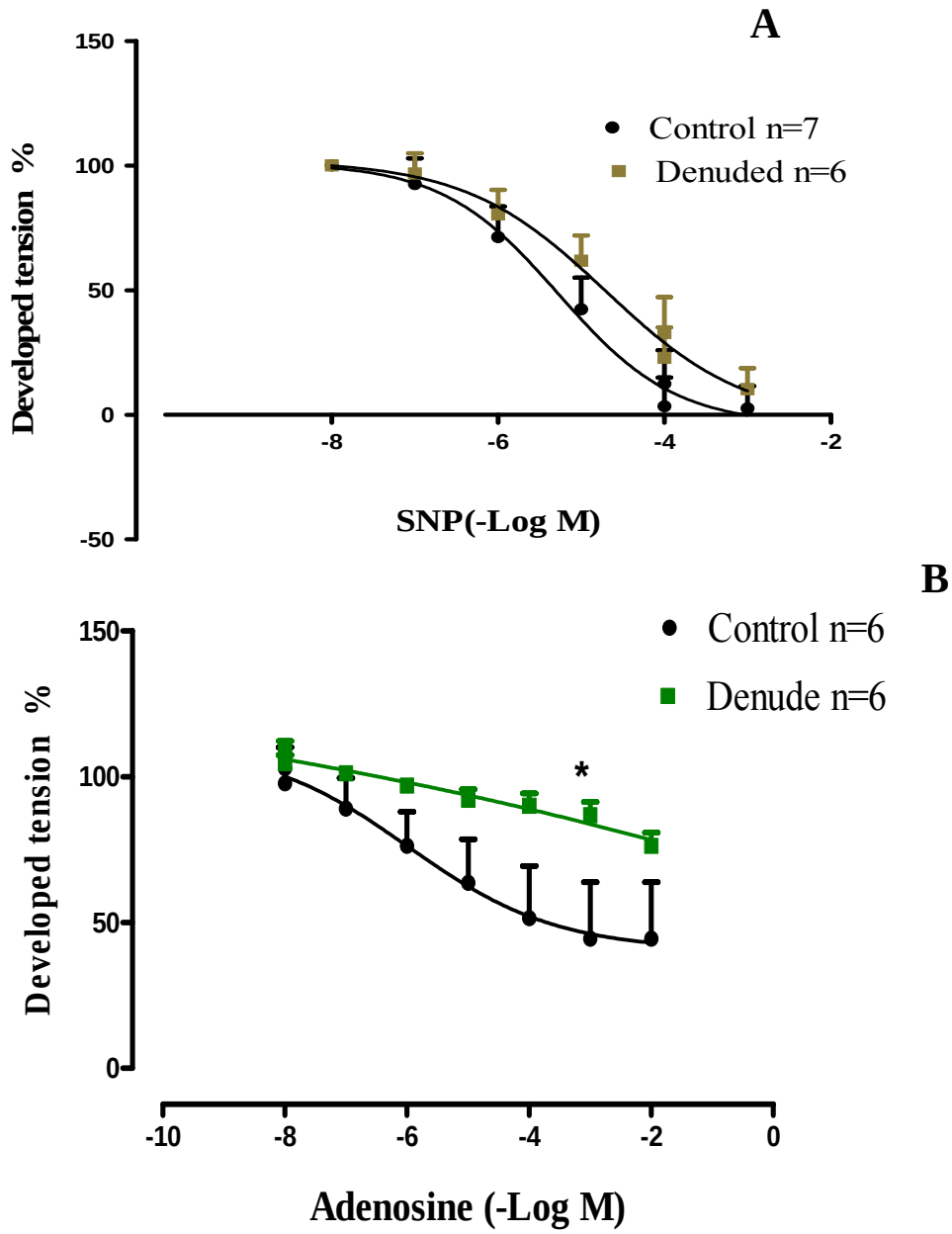

Fig 6. The effects of endothelium on the relaxant responses to SNP and Ado in PE precontracted rat aortic rings. (A and B). Dose response curve to SNP and Ado induced relaxation in control and in denuded endothelium aortic rings.

Discussion

In the present study, the mechanism of relaxation induced by SNP and Ado was further studied. To assess the role of K_{ATP} channel in SNP and Ado in inducing vasodilation, PE precontracted aortic rings were preincubated with GLIB showed disinhibiting vasodilation induced by SNP. This these indicate that SNP did not involved in opening of K_{ATP} channels to induce vasodilation.

To inhibit the synthesis of NO from L-Arginine in the presence of oxygen and by the help of NOS enzyme, L-NAME was used as NOS blocker. The current study showed that the NOS inhibitor effect of L-NAME did not attenuated or abolished SNP -induced vasorelaxation in rat's aortic smooth muscle. However the maximum relaxation for both SNP in presence of L-NAME slightly decreased as compared to control. This response may be due to direct dissociation of NO donor to produce NO without the action of NOS and also disexistance of exogenous precursor for NO. Therefore, any SNP induced changes in arteriolar diameter in the presence of L-NAME can be attributed to mechanisms independent on internal NO synthesis.

Nifedipine was used to test the role of NO blocking of calcium channel. The data of the current study showed that SNP significantly enhanced relaxation in smooth muscle. This is may be due to Ca^{2+} sensitization and the release of Ca^{2+} from one type of channel might increase the open probability of the other channel (Mayer et *al*., 2000 and Porter *et al*., 1998).

Active hyperporalization in rats aorta occurs when the release of NO from the endothelium is ongoing, so we examined the role of endothelium-derived NO in denuded endothelium rings induced vasorelaxation in the aorta using NO donors (SNP). Inhibition of the vasorelaxation induced by NO donors in rings with damaged endothelium not observed in the present study. This is may be due to direct dissociation of NO donor when added to solution without requiring to be released from endothelium. In the current study, our results indicated that K_{ATP} channels were not involved in SNP inducing relaxation in aorta with intact endothelium, while, the dilation response of aorta to Ado was attenuated by endothelial removal, suggesting that endothelial Adenosine receptors contribute in Ado vasorelaxation.

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کورتی:

سودیوم نایتروبروسایت (SNP) و ئەدینوسین (Ado) کاردکەن وەك دەرمانێن چالاك ل چارەسەرکرنا نەخوشین دلی. نایتریك ئوکساید (NO) دهێتە بەرهەم هێنان ل زوربەی خانێن دل و لوولەی و کاردکەن بۆ ڕێکخستنا کارێ لوولەی ب ڕێکخستنەکا هویر بۆ پروسێسا هاندان و کرژبونێ.Ado کو رولەکێ سەرەکی یێ هەی ل ڕێك و پێك کرنا شاخوێنبەری. ژ بەر هندێ ئارمانجا ڤێ ڤەکولینێ دیارکرنا بەشدارکرنا NO و Ado ل خاوکرنا ماسولکێن لوس ل شاخوێنبەری ب خانێن داپوشەری و بێ داپوشەرێ هاتینە جوداکرن ژ چوردێن سپی کو بەری هنگی هاتینە کرژکردن ب ڤینایلفرین.

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

و لدیف ئەنجامێن ڤێ ڤەکولینێ , GLIB خاوکرنا د پارچێن شادەماری ئەوا دروست بوی ژ لایێ Ado کێم کر (نرخێ خاوبۆنێ کێم کر ژ ٤١,٠٧ ± ٦,٧ کونترول بۆ ١٨,٥٤±٤,٦ ل پارچێن شادەمارێ هاتیە داینکر بGLIB .ئەف کیمکرنە هاتە بەروڤاژی کرن ل بەرسفا SNP بۆ خاوکرنا لووڵی پشتی شادەمار هاتیە داینکرن ب LNAM ی و بەرسفا SNP بۆ خاوبونی نەگرت، ژ لایێ دیڤە، نیفدپین ب شێوەیەکی باش خاوبون زێدەترکر ل شادەمارێهاتینە چالاك کرن ب چرێن جوراوجورێن SNPی. خاوبون بۆ Ado هاتیە کێمکرن نێزیکی (٦,٨٨± ١,٠١) پشتی داینکرنا پارچێن شادەمارێ داپوشەر ب کافاینێ, بس ب تمامی نە هاتە گرتن. لابرنا داپوشەری ژی بروسێسا خاوبونێ کێم نەبو بەروڤاژی بروسێسا خاوبونێ بلندکر.

الملخص:

نايتروبروسايد الصوديوم (SNP) والادينوساين (Ado) ادوية القوية يستخدم في علاج أمراض القلب والشرايين. ويتم إنتاج النيتريك كسيد (NO) تقريبا من جميع أنواع الخلايا الذين تتكون منهم القلب والأوعية الدموية وينظم وظيفة الأوعية الدموية من خلال التنظيم عملية الاستثارة و التقلص. Ado المادة الأيضية التي تلعب دورا رئيسيا في تنظيم ذاتي للشريان التاجي، ولذلك، كان الهدف من هذه الدراسة التحقيق في مساهمة NO و Ado في الاسترخاء حلقات العضلات الملساء الأبهر سليمة والمعرية البطانة المعزلة من الجردان, وتم استحداث تقلص باستخدام مادة phenylepherine (PE). تم عزل الشريان ابهر ، وقطع إلى حلقات، وربط في الجهاز organ bath. تم تسجيل شدة العضلة باستخدام powerLab 870 Model ML) Data Acquisition System).

وفقا لنتائج هذه الدراسة، انخفضت استجابة الاسترخاء الناجم عن Ado في حلقات الأبهر المحضنة مع Glybenclamide (GLIB) (معدل انخفاض قيمة توسع الأوعية ٤١,٠٧± ٦,٧ في Control و١٨,٥٤ ± ٤,٦ في حلقات الأبهر المحضنة مع GLIB). L-nitroarginine methylester (L-NAME), لم تلغ الارخاء الناجم عن SNP، في ناحية أخرى نيفيديبين ادت الى زيادة كبيرة في الارخاء الناجم عن SNP بطريقة تعتمد على الجرعة في حلقات سليمة البطانة. كافاين قلت الاسترخاء الناجم من (Ado) في حلقات الأبهر سليمة البطانة ولكن لم تلغ تماما (٦,٨٨ ± ١,٠١) مقارنة مع الكنترول. من ناحية أخرى، إزالة البطانة في حلقات الأبهرية لم يخفف عملية الارخاء الناجم عن SNP. بينما، قلت جزئيا الارخاء الناجم من Ado في حلقات الأبهر العارية من البطانة. هذه النتائج اضهرت أن (١) قناة البوتاسيوم معتمد علىATP لم تشارك في ارتخاء وعائي الناجم من SNP ، في حين يكون له دور في vasorelation بوساطة Ado. (٢) ارتخاء الناجم من NO لا يعتمد على البطانة، في حين، بطانة لها تاثير جزئى على Ado.