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Mechanisms Underlying the Relaxation Induced by Bradykinin in Rat Aorta

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ABSTRACT

Bradykinin (BK) plays a critical role in intervening relaxation in vascular smooth muscle through distinctive mechanisms. The current project is designed to study the role of PKA, calcium-dependent potassium (BKca, IKca,andSKca) channels, voltage-dependent potassium channel (Kv) and inward rectifier potassium channel (Kir) in BK interceded response and to determine functionally the mechanisms underlying BK mediated relaxation, utilizing standard tissue bath protocols. The results of the current work showed that BK inducing vasorelaxation is mediated by PKA which activate eNOS (endothelial nitric oxide synthase) followed by a subsequent release of nitric oxide (NO) from endothelial cells since the protein kinase inhibitor (H-89) significantly reduced E_{max} and pIC50. Similarly, in rings pre-incubated with L-NAME (3×10^{-4}M) significantly inhibited vasorelaxation induced by BK. Furthermore, both Kca and Kv played an interesting role in vasorelaxation mediated by BK (1×10^{-6} to 3×10^{-3} M) in phenylephrine (PE) precontracted aortic rings, pre-incubated with tetraethylammonium (TEA) which reduced E_{max} with a slight reduction of pIC50 as compared to the control. Also, aortic rings pre-incubated with IK(Ca) blocker (clotrimazole) markedly reduced E_{max} but with a tendency of pIC50 elevation. In the present study, voltage-dependent potassium channels blocker (4-AP) significantly reduced the relaxant effects of BK and reduced E_{max} with altering pIC50 values significantly. On the other hand, $BaCl_2$ tends to enhance the relaxation induced by BK and significantly elevated E_{max}. Collectively, our findings indicate that BK-induced relaxation is dependent on the activation of PKA to release NO from endothelium which through a cascade of a signaling pathway, in turn, activate both IK(Ca) and Kv in rat artic vascular tissue.

Keywords: Bradykinin, Nitric oxide, Hyperpolarization, Endothelium, Organ bath, PowerLab system, Aorta

INTRODUCTION

Bradykinin is the endogenous peptides produced by the vascular kinin-kallikrein system. The local hormones kinins enhance the release of endothelium-derived relaxing factors (EDHF) such as NO and prostaglandins, which helps in the relaxation or contraction of smooth muscle and elevate vascular permeability, adhesion of molecule expression and mitogenesis [1,2]. The biological impacts of BK are interceded by the incitement of particular receptors, classified as B1-R and B2-R [3]. The B2-R showed to be capable for the majority of kinins actions *in vivo* impacts cardiovascular physiology, counting expanded vascular penetrability, endothelium-dependent vasodilatation, vascular and bronchial smooth muscle compression [4]. B1-R is inducible and applies their activities in response to tissue damage, aggravation, endotoxin, anoxia, myocardial dead tissue and proinflammatory cytokine [5]. The major mechanism responsible for BK-stimulated NO production is at least partly intervened by eNOS phosphorylation [6]. Few studies indicated that BK-mediated dilation of canine coronary arteries decreased after inhibition of NO synthesis, whereas others indicated that in porcine coronary arteries, inhibition of NO synthesis has only minor effects on BK-induced dilation [7].

The classical G(q) protein-coupled receptor activation of phospholipase C-β (PLC-β) after stimulation of B2-R by BK initiates the formation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), driving to a biphasic increment in free ($[Ca^{2+}]i$). The PLC-β is thought to be involved in the activation of transient receptor potential (TRPC) channels.

Seven members of mammalian TRPC exist designated as TRPC1-TRPC7, and in some endothelial cells, BK stimulates TRPC6 to induced increases in intracellular [Ca^{2+}]i. Phosphorylation of protein kinase A at serine1179 is another mechanism by which BK activates eNOS [8]. A similar response in uterine artery of the guinea pig, human coronary artery and small mesenteric arteries of the rat has been documented [9], while other suggestions have revealed that dephosphorylation of eNOS-Thr497 participated in BK-stimulated NO production [2].

The purpose of the current study is to explain the mechanisms of BK-induced vasorelaxation in smooth muscle of rat aorta pre-contracted with PE in intact and endothelium-denuded rings with a special emphasis on the roles of different K+ channel subtypes and PKA to characterize functionally the mechanisms underlying BK-induced relaxation by using several ion channels and enzyme inhibitors.

PATIENTS AND METHODS

Tissue Preparation

Male albino rats *Rattusnorvegicus* weighing (200-270 g) were used in 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 Ethics Committee at the University of Zakho. The animal was injected intraperitoneally with heparin (2000 units/200 g) for several minutes to avoid blood clotting and aortic endothelium damage. After anesthetization, the descending thoracic aorta was isolated smoothly without any stretching force and the intact aorta was immediately transferred to Krebs bicarbonate solution (136.9 mMNaCl, 5.4 mMKCl, 5.5 mM D-(+)-Glucose, 23.8 mM$NaHCO_3$, 1 mM$MgCl_2.6H_2O$, 1.5 mM$CaCl_2.2H_2O$). The aorta was cleaned from periadventitial tissue in cold Krebs solution and was cut transversally into ring segments (each of 2-2.5 mm in length) [10].

The rings were placed in a 10 ml glass tissue chamber containing Krebs solution with constant aeration of about 95% oxygen and 5% CO_2, pH=7.3, maintained at 37ºC. Two stainless-steel wires were passed through the lumen of each ring, one stirrup was connected to the base of the tissue chamber whereas the other was connected to an isometric force transducer (Model FORT100) to measure tension in the vessels and was connected to a PowerLab data acquisition system (Model ML845, AD Instruments, Australia). 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, then allowed to equilibrate for 1 hr in the physiological solution and were washed after every 15-20 minutes [11]. In endothelium-denuded experiments, the wall of the vessel was subjected to a gentle rubbing by a syringe needle covered with a piece of cotton and the integrity of endothelium was assessed qualitatively by the degree of relaxation caused by acetylcholine (10 μM) after precontraction with PE. If relaxation with acetylcholine was not ≥ 80%, the ring was discarded. In experiments with endothelium-denuded vessels, if there was any degree of relaxation the rings were discarded.

Evaluation of the Mechanisms Underlying the Relaxant Effect Induced by Bradykinin

Endothelium-intact and denuded vessels were precontracted with (1mM) PE. After stable and sustainable pre-contraction, the rings were treated cumulatively with BK at concentrations ($1\times10^{-6}$to 3×10^{-3}M). To test the role of potassium channels subtypes in the development of relaxation induced by BK, aortic rings were preincubated with the (1 mM) of TEA, 4-AP, and $BaCl_2$, (1×10^{-5}M) of clotrimazole. To examine the effect of interaction between two types of potassium channels, TEA and 4-AP were used. To test the effect of blocking of NO in the presence of BK, the aortic

rings were preincubated with L-NAME (3×10^{-4}M). In all the experiments, the aortic rings were preincubated with the desired drugs for 30 minutes before their precontraction with PE.

Statistical Analysis

Results are expressed as means ± SEM. Agonist dose-response curves were fitted using a non-linear interactive fitting program (Graph Pad Prism 6.0 (Graph Pad Software, USA). Potency (pIC50=-Log IC50) which is used as a standard to compare the potencies of vasoactivator substances. The maximum relaxant effect (E_{max}) was considered as the maximal amplitude response reached in concentration-response curves for both relaxant agents. For comparison between means of two groups, two ways ANOVA and Bonferroni test were used. The value of $p<0.05$ were considered statistically significant.

RESULTS

The relaxation induced by BK was inhibited after preincubation of aortic rings with H-89. The maximum relaxation value significantly reduced from 60.79 ± 1.189 to -11.8 ± 2.754 as compared to the control. While pIC50 tended to decrease from 4.180 ± 0.073 in the control to 1.650 ± 0.56 in H-89 preincubated aortic rings. At low concentration of BK, H-89 enhanced vasoconstriction, followed by small decreasing in the vasoconstriction but then no further increase in vasorelaxation was observed. To find out the role of NO signaling pathways in BK-induced vasorelaxation, the inclusion of aortic rings with L-NAME significantly inhibit vasorelaxation induced by BK with an E_{max} -15.2 ± 0.614 and pIC50 4.101 ± 0.012 as shown in Figure 1 and Table 1.

Table 1 E_{max} ± SE % and pIC50 ± SE of BK-induced vasorelaxation in rat aortic rings pre-incubated with several inhibitors

Inhibitors	E_{max}± SE (%)	pIC50± SE
Control	60.79 ± 1.189	4.180 ± 0.073
H.89	-11.8 ± 2.754	1.650 ± 0.56
L-NAME	-15.2 ± 0.614	4.101 ± 0.012
TEA	41.55 ± 5.204	3.717 ± 0.041
Clotrimazole	- 3.5 ± 0.226	4.462 ± 0.086
4-AP	8.5 ± 2.798	2.671 ± 0.018
BaCl2	119.803 ± 3.115	2.875 ± 0.032
TEA + 4-AP	35.38 ± 1.455	2.773 ± 0.033
Endothelium Denuded	-33.99 ± 4.181	6.423 ± 0.013

In the current study, E_{max} reduced in aortic rings preincubated with TEA (1 mM) for 30 minutes before cumulative addition of different BK concentrations, but was not blocked completely as compared with the control 41.55 ± 5.204 vs 60.79 ± 1.189 with PIC50 3.717 ± 0.041 and 4.180 ± 0.073, respectively (Figure 1, Table 1). Also, IK(Ca) channel plays an important role in the relaxation response induced by different concentrations of BK, since clotrimazole significantly inhibited the E_{max} to -3.5 ± 0.226, however it slightly increases PIC50 to 4.462 ± 0.086 as compared to the control (Figure 2, Table 1).

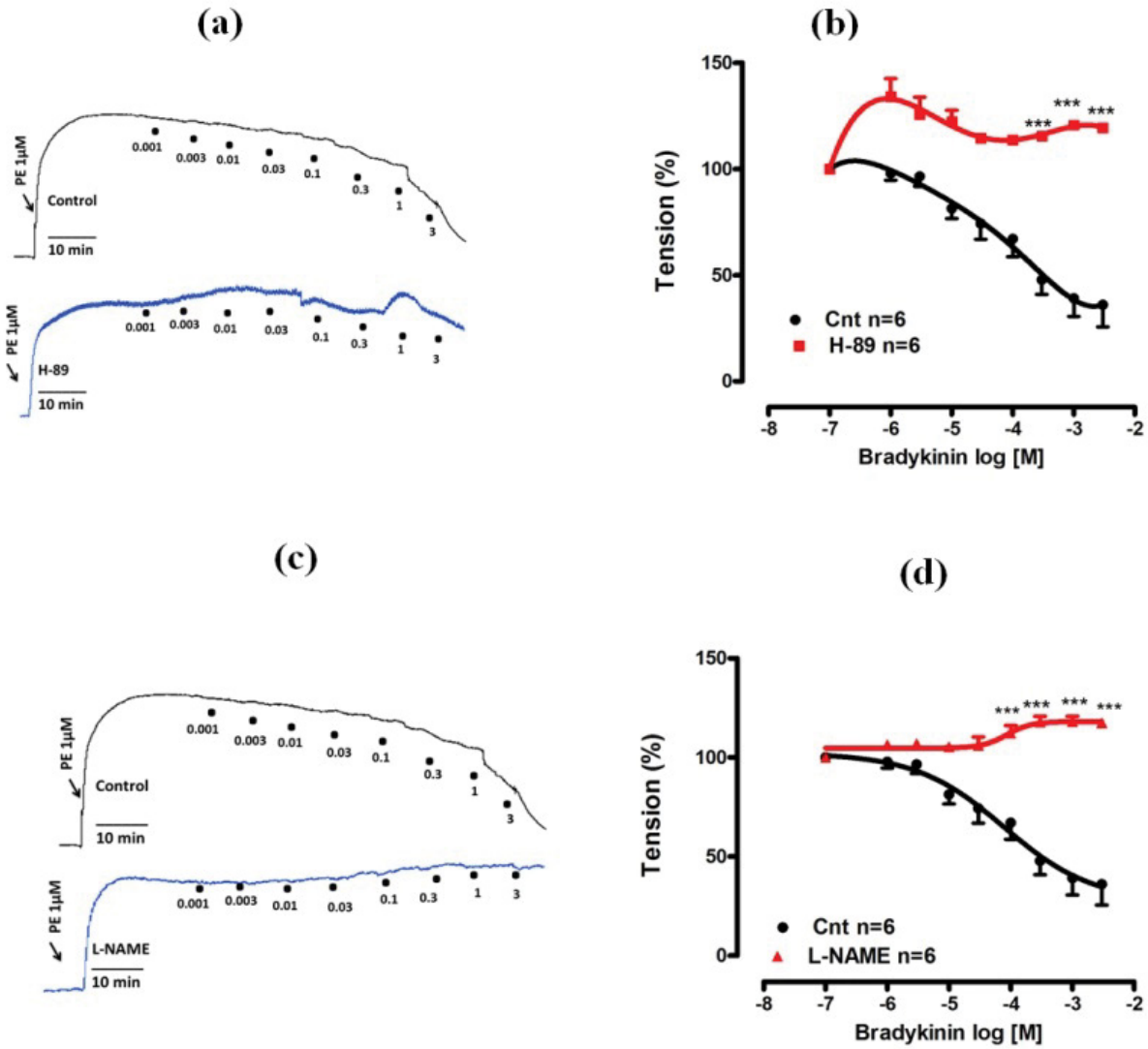

Figure 1 Effects of different concentrations of BK on aortic rings precontracted with PE (1 µM) (a) Typical chart view trace, (b) Dose-response curves showing comparative vasorelaxation effects of BK on PE-induced vasoconstriction (control) and aortic rings preincubated with H-89, (c) Typical chart view trace and (d) Dose-response curve showing comparative vasorelaxation effects of BK on PE-induced vasoconstriction (control) and aortic rings preincubated with L-NAME. In the chart, trace indicates the addition of BK (mM) in a cumulative manner and for each dose 3 min

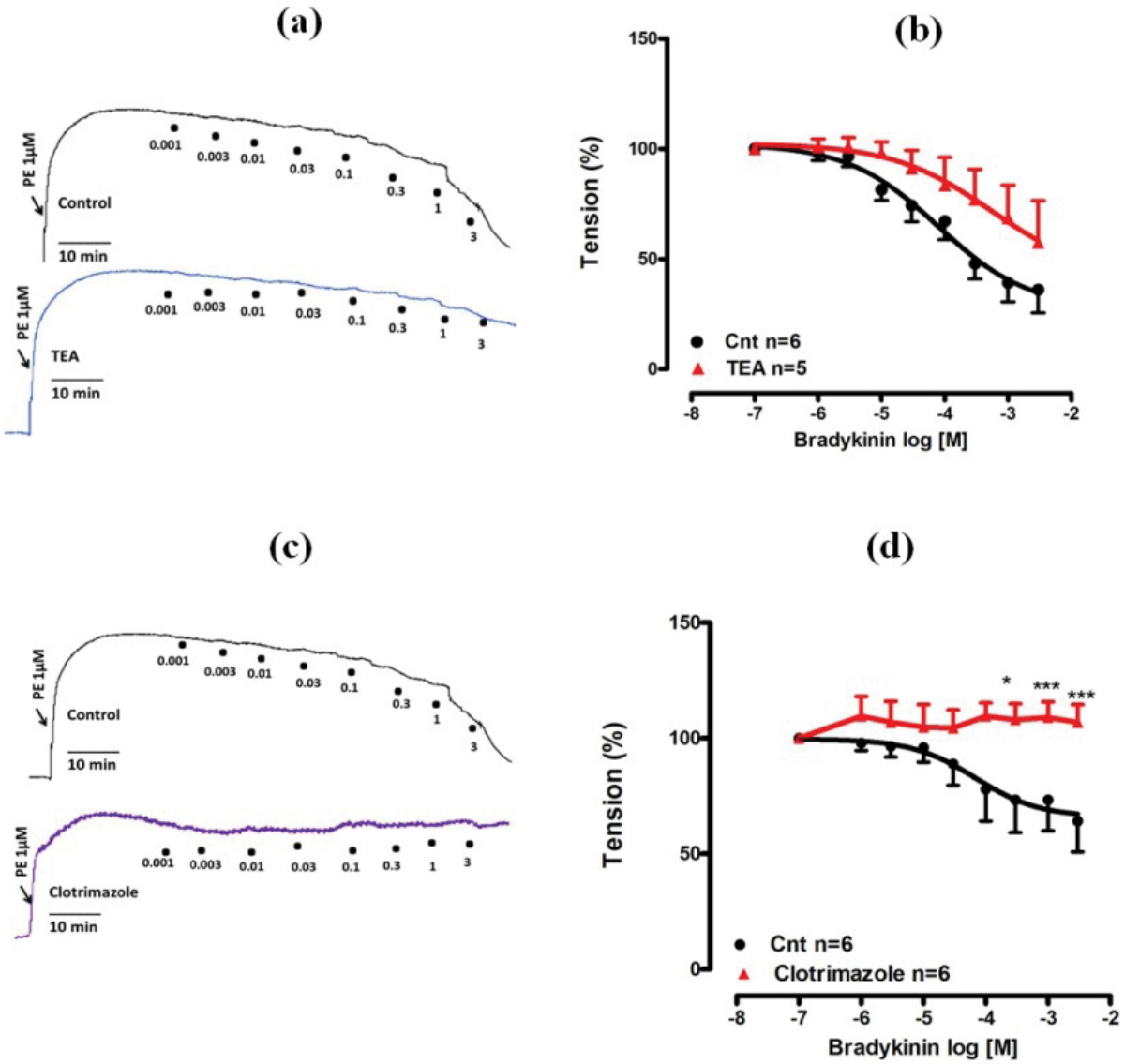

Figure 2 Effects of different concentrations of BK on aortic rings precontracted with PE (1 µM) (a) Typical chart view trace, (b) Dose-response curves showing comparative vasorelaxation effects of BK on PE-induced vasoconstriction (control) and aortic rings preincubated with TEA, (c) Typical chart view trace and (d) Dose-response curve showing comparative vasorelaxation effects of BK on PE-induced vasoconstriction (control) and aortic rings preincubated with clotrimazole. In the chart, trace indicates the addition of BK (mM) in a cumulative manner and for each dose 3 min

The current results revealed that the response induced in aortic rings by BK significantly blocked on preincubation with 4-AP, which clearly reflects the role of Kv channels in BK-induced relaxation. The E_{max} decreased to 8.5 ± 2.798 as compared to the control, while pIC50 was markedly decreased from 4.180 ± 0.018 in the control to 2.671 ± 0.073 in rings preincubated with 4-AP. On the other hand, $BaCl_2$ enhanced vasorelaxation mediated by BK, initially at low doses of BK (10^{-6} to 3×10^{-5}M), then slightly increase contraction followed by gradual relaxation and ultimately by a sharp relaxation at higher concentrations of BK and E_{max} reached to 119.803 ± 3.115 (Figure 3 and Table 1).

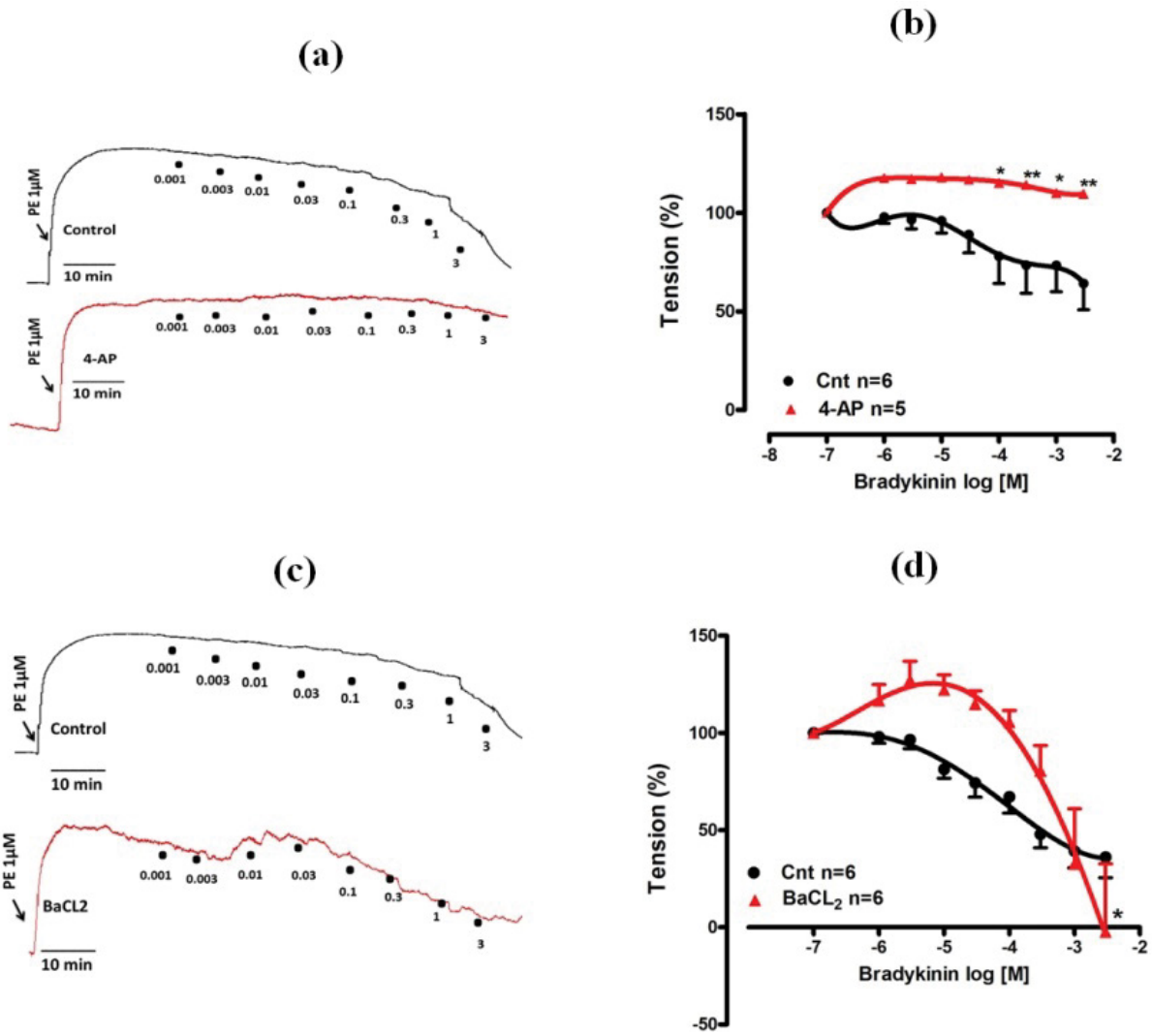

Figure 3 Effects of different concentrations of BK on aortic rings precontracted with PE (1 µM) (a) Typical chart view trace, (b) Dose-response curves showing comparative vasorelaxation effects of BK on PE-induced vasoconstriction (control) and aortic rings preincubated with 4-AP, (c) Typical chart view trace, and (d) Dose-response curves showing comparative vasorelaxation effects of BK on PE-induced vasoconstriction (control) and aortic rings preincubated with BaCl2. In the chart, trace indicates the addition of BK (mM) in a cumulative manner and for each dose 3 min

A combination of TEA and 4-AP showed inhibitory effect on BK-induced vasorelaxation which was reduced more than that induced by TEA alone and less than that induced by 4-AP alone. Thus, the E_{max} reduced from 60.79 ± 1.189 in control to only 35.38 ± 1.455. Relaxation in endothelial denuded rings was significantly reduced as compared to the control, and the E_{max} value was -33.99 ± 4.18. However, it markedly increases pIC50 (Figure 4 and Table 1).

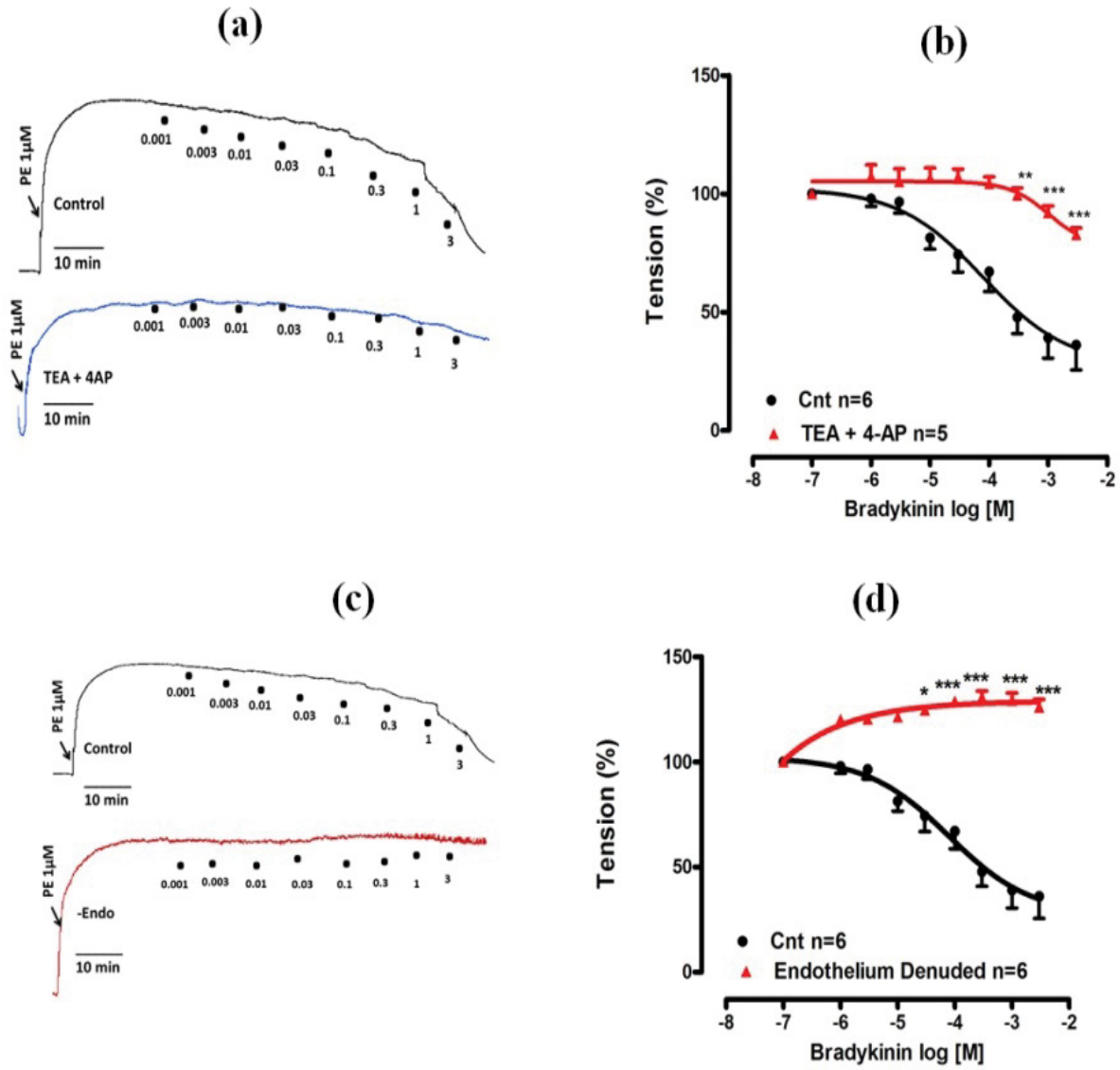

Figure 4 Effects of different concentrations of BK on aortic rings precontracted with PE (1 µM) (a) Typical chart view trace, (b) Dose-response curves showing comparative vasorelaxation effects of BK on PE-induced vasoconstriction (control) and aortic rings preincubated with Tea+4-AP, (c) Typical chart view trace, and (d) Dose-response curves showing comparative vasorelaxation effects of BK on PE-induced vasoconstriction (control) and Endothelium denuded aortic rings. In the chart, trace indicates the addition of BK (mM) in a cumulative manner and for each dose 3 min

DISCUSSION

The functional regulation of vascular tissue is critically relying on the endothelium-derived NO and endothelium-derived hyperpolarizing factors (EDHF). Various stimuli such as shear stress, Bradykinin, and receptor-operated agonists stimulate endothelium eNOS to release nitrous. The results of the current study showed that BK-induced relaxation was mediated by PKA activation which phosphorylates eNOS. These observations are in agreement the findings made by Tirapelli, et al., in 2009 who revealed that BK stimulates B2-R in endothelial cell culture as well as in mouse carotid arteries and release NO to regulate the vascular muscle tone [12].

The data of the current study indicate that maximum relaxation value induced by BK decrease after incubation with H-89 as compared to control, but it was not completely abolished. This finding indicates that in addition of PKA, another unknown protein phosphates involved in BK-induced vasorelaxation such as PKG, therefore the molecular pathway underlie the role of protein phosphates in eNOS activation by BK remain to be clarifying.

The resulted inhibition of NO synthase by L-NAME abolishes vasodilatory effect induced by BK, suggesting that BK effect was mediated via endothelial NO release. This data was in agreement to Tirapelli, et al., who reported that vasorelaxation induced by BK modulates vascular smooth muscle tone, is abrogated in the presence of L-NAME in pig's carotid arteries [12]. Elevation of [Ca^{+2}] by BK is necessary to release NO, which activates VSMCs guanylate cyclase, increasing intracellular cGMP, thus causing relaxation through reducing of [Ca^{+2}]i and activation of K+ channels [13-15]. Furthermore, NO itself activatesKCachannels to induce hyperpolarization in VSMCs [16,17].

It is worthwhile to mention that for the first time it has been indicated that both IK(Ca) and Kv have an important role in BK-induced vasorelaxation in rat aorta. The results show that incubation of aortic rings with TEA partially inhibits vasorelaxation induced by BK, whereas application of clotrimazole completely abolished the relaxation response. This indicates that KCahasa role in BK-induced vasorelaxation particularly IK(Ca) since a maximum inhibition was observed in the presence of clotrimazole. Studies showed that BK in whole patch clamp, the outward current that was produced by BK inhibited about 75% by charybdotoxin [18]. Bradykinin-mediated relaxation reduced in aorta pretreated with 4-AP, interestingly and according to our results, the roles of Kv channel to be taken with consideration in BK-induced vascular relaxation. It has been suggested that the transient increase of cytosolic Ca^{2+}, activate calcium channels in the plasma membrane to influx Ca^{2+} from extracellular and to produces depolarization which in turn activate Kv to hyperpolarize membrane and facilitate smooth muscle relaxation [19-21].

Pre-incubation of aortic rings with $BaCl_2$ did not inhibit the BK dependent relaxation. This finding showed that Kir channel played a limited role in BK actions. It has been demonstrated that in forearm blood flow, BK response was partially, but not entirely, inhibited by $BaCl_2$ [22]. Previously, several studies have indicated that BK may activate Kir channels in human coronary micro arteries and coronary arterioles [23,24].

The results of the current study clearly indicated that endothelium integrity is an essential prerequisite for BK-induced relaxation. This was confirmed by the results from this study that indicated inhibition of BK mediated vasorelaxation in endothelial denudation aorta. The role of endothelium inBK-induced relaxation has been described in various vascular tissues such as bovine middle cerebral arteries and porcine coronary arteries [25]. However, this has been described for the first time in rat's aorta. This indicates the participation of endothelial B2 receptor in relaxant effect of BK which was further supported by the results on dog carotid artery [26].

CONCLUSIONS

The results of the present study showed that BK-induced relaxation to rely on its stimulatory effect on endothelial cells to activate PKA, which in turn activates eNOS to release NO. BK-induced relaxation depends mainly on the activation of KCa specially IK(Ca) and Kv channels with the limited role of Kir channel. This study showed for the first time that Kv channel plays an important role in the BK-induced response.

DECLARATIONS

Conflict of Interest

The authors declare no potential conflict of interest.

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