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THE ROLES OF CYCLOXYGENASE AND ENDOTHELIAL DERIVED HYPERPOLARIZING FACTORS IN BRADYKININ-INDUCED AORTIC RELAXATION

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ABSTRACT:
The present study is designed to investigate the roles of cycloxygenase (COX) and endothelial derived hyperpolarizing factors (EDHF) pathways in bradykinin (BK)-induced aortic relaxation. Here, isolated aortic rings pre-incubated with different ion channel blockers which are; inward rectifier potassium channel blocker (barium chloride; $BaCl_2$), calcium activated Potassium (K_{Ca+2}) channel blocker (tetraethylammonium; TEA), cytochrome P450 inhibitor, clotrimazole and cycloxygenase inhibitor and indomethacin. In $BaCl_2$, E_{max} tended to decrease significantly with significant change of PIC50. TEA pre-incubation markedly shifted DRC of BK to the left side and it significantly reduced PIC50. Indomethacin significantly lowered the PIC50 of BK, but it shifted the DRC of BK to the left. The results suggested that BK relaxes aortic smooth muscle particularly via the enhancement of cycloxygenase and epoxygenase enzymes as well as through opening K_{ir} and $K_{Ca}{}^{+2}$channels.

KEYWORDS: Bradykinin, Aorta,cycloxigenase ,epoxygenase , Kir and KCa+2channels channels.

1. INTRODUCTION

Kallikrein-kinin system (KKS) was discovered in 1928 by Frey and coworkers; they extracted it from snake venom (Costa-Neto et al., 2008). The KKS consists of precursor kininogens, kallikrein and the kinin peptides. Kallikreins are belonging to serine proteases group, which convert kininogens to kinin. The nonapeptide BK considered as potent vasoactive peptide that is formed in the plasma by cleavage of a high-molecular-weight kininogen. Bradykinin exerts its physiological effects through two G-protein-coupled receptors, termed the B 1 and B 2 receptors (Tirapelli, Bonaventura, Tirapelli, & de Oliveira, 2009). In endothelial cells, BK through Ca^{2+}-mediated mechanisms stimulates endothelial nitric oxide synthase (eNOS) and increased production of nitric oxide (NO) and cGMP, that results in vasorelaxation in the vascular bed (Jagdish N Sharma, 2013). According to an *in vitro* study, BK induce a vasodilator response in rats aortic ring (Fukada, Tirapelli, de Godoy, & de Oliveira, 2005). In cardiovascular system, B2 receptors activation shown to antiarrhythmic effect on the heart and antithrombotic in the vasculature (J. N. Sharma & Al-Sherif, 2011).

Prostaglandins play an important role in determining the sensitivity of gastrointestinal afferents to BK. There is review suggested that BK acts through B2 receptors to stimulate prostaglandin E2 (PGE2) release, which in turn sensitizes serosal afferent nerve endings to a more direct action of BK at B2 receptors. BK increases PGs production in different ways. BK leads to the Ca^{2+}-dependent and Ca^{+2}-independent phosphorylation of cytosolic phospholipase A2 (cPLA2). The Ca^{+2}-dependent and Ca^{+2}-independent A2 liberate arachidonic acid from membrane phospholipids. Furthermore, BK leads to the induction of cyclooxygenase-2, which converts arachidonic acid into PGs (Kakoki & Smithies, 2009).

Badykinin and the Ca^{+2}ionophore A23187 cause the release of EDHF in the human coronary arteries. Since this endothelium-dependent hyperpolarization is present in coronary arteries from patients with different cardiac diseases (Nakashima, Mombouli, Taylor, & Vanhoutte, 1993), it was stated that BK-induced causes vasodilation through activation of the B2 receptor and subsequent release of NO, prostacyclin and EDHF (Rahman et al., 2014). EDHF contributes in dilation of microvessels which significantly reduces vascular resistance (Honing, Smits, Morrison, & Rabelink, 2000).

Epoxyeicosatrienoic acids (EETs) are cytochrome P450 metabolites of arachidonic acid that are produced by the vascular endothelium in response to agonists such as BK, acetylcholine or physical stimuli such as shear stress or cyclic stretch in the vasculature. The EETs have biological actions that are involved in the regulation of vascular tone, hemostasis and inflammation. Bradykinin, acetylcholine, and shear stress cause endothelium-dependent hyperpolarization which is blocked by cytochrome inhibitors and K channel blockers (Campbell & Fleming, 2010). Because of little is known regarding the actions of BK on vascular tone, therefore the main aims of the present study were to investigate the role of COX and EDHF pathways in BK-induced aortic relaxation.

2. MATERIALS AND METHODS

2.1 Experimental animals

Male albino rats (310-350 gm) were used in the current study, rats were housed and maintained in a temperature controlled room (22±4)ºC. Animals were given standard rat diet and tap water *ad libitum*. They were exposed to 12 hrs light/dark cycle and fasted in about (10 hrs) before experimentation.

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2.2 Preparation of rat aortic rings

Albino male rats were anaesthetized with intra-pritoneal injection of ketamine (40 mg/kg) and xylasine (10 mg/kg) followed by opening the thorax and isolation of the thoracic aorta from the dissected rat as quickly as possible without stretching and any mechanical force to obtain intact aorta. The aorta was transferred to a petri-dish filled with ice cold Krebs-Henseleit solution aerated with about 95% oxygen in 37ºC. After removing the adherent connective tissues, the aorta was cut into each with 3-4 mm long which was put into Krebs solution immediately.

2.3 Isometric tension recording in isolated vessel

The aortic ring was held up by two stainless steel clamps (Tissue clamp, Model Le 0140, Panlab Harvard apparatus, USA). One of the clamps attached to a hook at the bottom of the glass tissue chamber, and the other was connected to the force transducer through a thread to record vasoreactivity from the aorta to transducer it by amplifier (Quad bridge amplifier Powerlab 8/35) (Fig 3.4). Isometric tension was detected by the transducer which is connected to the amplifier when the smooth muscle of the aorta ring contracts or relax, and the data was recorded by data acquisition software (LabChart 7.1). The held up aortic ring was immersed in Krebs solution contained in a 10 ml organ chamber. Krebs solution was maintained at pH 7.4 and was constantly aerated with carbogen in about 95% oxygen 5% carbon dioxide at 37ºC (LE 13206 Thermostat, Panlab Harvard apparatus, USA).

The aortic rings were tensed to a stable basal strain of two gm before left to equilibrate for one hour. Krebs solution was replaced at 15-20 minutes intervals in the bath chamber. After stabilization, the viability of the aorta was tested to be sure that the aorta is still alive or not by pre-contraction with KCl 60 mM (EC_{50}, Dose response curve previously obtained in laboratory). In order to reach stability the aorta washed many times and the experimental substances introduced into bath chambers according to the protocols, the aorta incubated with drugs for 20 minutes then PE 10^{-6} M (Dose response curve previously prepared in laboratory) until reaches maximum contractility and plateau, then relaxation occurred by dose response curve for BK ($1X10^{-10}$ - $1X10^{-5}$ M) and cumulative –dose response obtained in time interval of four minutes.

2.4 Experimental design

The present study included the following groups:

2.4.1 Group 1: Control (n=8): After stabilization, the aorta was pre-contracted with PE (10^{-6} M), and when the peak response reached the plateau, the cumulative concentrations of BK (10^{-10}-10^{-5}) was added every four minutes.

2.4.2 Group 2: Kir channel blocker (n=8): The aorta incubated with $BaCl_2$ for 20 minutes, then contracted by PE and relaxed BK doses, to identify the role of K_{ir} channels in the vasodilatory mechanism of BK.

2.4.3 Group 3: Tetraethylammonium (n=6): The aorta pre-incubated with TEA (1 mM) for 20 minutes, then contracted with PE then relaxed by BK.

2.4.4 Group 4: Indomethacin (n=6): The aorta incubated with indomethacin (10^{-5} M) for 20 minutes, then contracted by PE and relaxed by BK doses.

2.4.5 Group 5: Clotrimazole(n=6): The aorta pre-incubated for 20 minutes with clotrimazole (30 mM), then contracted by PE and relaxed by BK doses, and pre-incubated for 20 minutes.

2.5 Statistical analysis

The concentration of the BK producing IC50 was determined after logarithmic transformation of the normalized concentration– response curves and is reported as the negative logarithm (-log IC_{50} = pIC_{50} values) of the mean of individual values, using Graph Pad Prism version 6.0 (Graph Pad Software Inc., San Diego, CA). Results were expressed as means ± SE and the values were compared by ANOVA and a post hoc Bonferroni's multiple-comparison test was carrying out pair wise comparison between the same doses of different groups and determine significance among groups, Values were considered to be significantly different when $P < 0.05$.

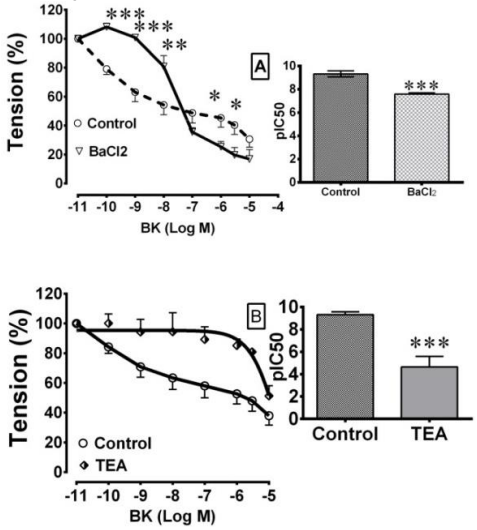

Figure 1. Cumulative dose-response curves for BK in producing relaxation of isolated aorta pre-contracted with 10^{-6}M PE. This shows comparative vasorelaxation effects of BK with $BaCl_2$, A) and TEA,B).

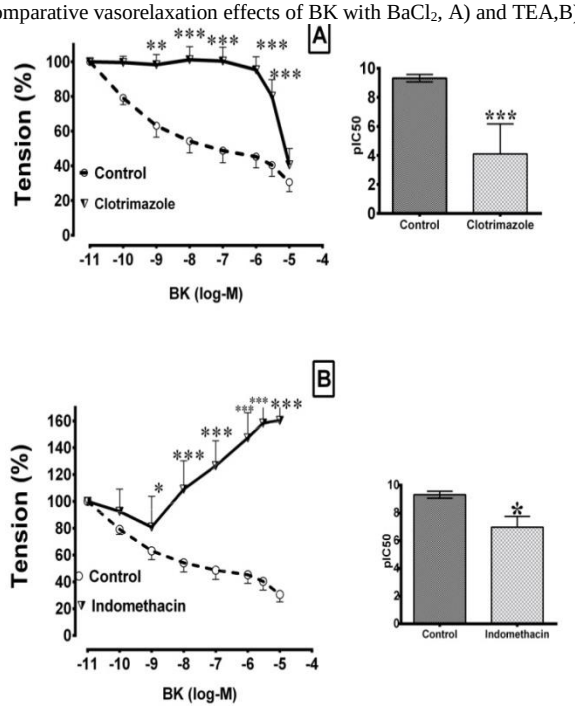

Figure 2. Cumulative dose-response curves for BK in producing relaxation of isolated aorta pre-contracted with 10^{-6}M PE. This shows comparative vasorelaxation effects of BK with Clotrimazole, A) and Indomethacin, B).

3. RESULTS

As shown in table 1 and figure 1 A, E_{max} tended to decrease significantly from (95.92±5.802) of BK to (83.2±2.853) of $BaCl_2$ with a significant change in PIC50 value. Figure (1.B) shows TEA pre-incubation which markedly shifted DRC of BK to the right side and it significantly reduced PIC50.
Indomethacin significantly changed PIC50 of BK and shifted the DRC of BK to the right. Also, E_{max} elevated in indomethacin as shown in table 1 and figure (2.B). Results viewed in figure (2.A) revealed that clotrimazole pre-incubation decreased E_{max} from (95.92±5.802) of BK to (59.47±2.966) of clotrimazole significantly. Nevertheless, it significantly reduced PIC50 of BK and slightly shifted the DRC to the right.

Table 1. Effect of $BaCl_2$, TEA, Indomethacin and clotrimazole on pIC50 and E_{max} in BK produced relaxation of isolated aortic rat rings.

Treatment	pIC50	E_{max}
Control	9.322±0.2528	95.92±5.802
$BaCl_2$	7.593±0.1057 ***	83.2±2.853
TEA	4.647±0.9385	48.73±3.068
Indomethacin	6.979±0.7703*	162.9±24.30
Clotrimazole	4.107±2.060***	59.47±2.966

The maximum relaxant effect (E_{max}) was considered as the maximal amplitude response reached in concentration–effect curves for both relaxant agents. Results are expressed as means ± SE and the values of pIC50 and E_{max} in $BaCl_2$, TEA, indomethacin and clotrimazole are compared with control (BK) group.

4. DISCUSSION

The present finding revealed that even low dose of $BaCl_2$ significantly reduced the vasorelaxant effects of BK. This indicates those k_{irs} channels involve are in the smooth muscle relaxation via cell membrane hyperpolarization (Schubert et al., 2004)**.** Moreover, the vasoconstrictor effect of $BaCl_2$ due to a fact that $BaCl_2$ interfere with BK potentiated EDHF vasodilatory mechanism. (Madeddu, Emanueli, & El-Dahr, 2007) concluded that the endothelial-dependent vasorelaxation by kinins includes activation of NO independent ion channels located in vascular smooth muscle cells (VSMCs) that account for endothelium-dependent hyperpolarization. One possible mechanism of this finding is that the hyperpolarization of endothelial cells regulated by activation of CYT P450 and resulting generation of epoxyeicosatien EETs (Busse et al., 2002)**,** however the exact mechanism of this BK induced relaxation needs further confirmation. Pre-incubation with TEA produced a tremendous inhibition in BK-induced relaxation. This has been explained on the bases of BK activates K^+ efflux through K_{Ca2+} involving both BK_{Ca} and SK_{Ca} (Liu, Freyer, & Hall, 2007), and such activation are suppressed by TEA pre-incubation. Recently, (Cuddapah, Turner, Seifert, & Sontheimer, 2013) demonstrated that K_{Ca2+} channels are activated as a result of BK-dependent Ca^{2+}increase.The main reason behind the vasoconstriction effect of TEA is that the muscle is under depolarization and Ca^{+2} enter the cells while $BK_{Ca}{}^{+2}$ channel opens during hyperpolarization and cause relaxation (Sanchez & López-Zapata, 2011).

Indomethacin significantly changed PIC50 of BK and shifted the DRC of BK to the right. The present finding consistent with the fact that BK leads to induction of COXs, which converts arachidonic acid to PGs (Kakoki & Smithies, 2009). Also, recently it has been established that BK strongly participates in the regulation of vascular tone by releasing some vasodilator factors inducing prostacyclin (Nishijima et al., 2014). Stimulation of PGs mostly occurs through activation of (eNOS) by BK B2 receptor, thereby increases cytosolic Ca^{+2} sensitive isoforms of phospholipase (Madeddu et al., 2007).

The present results revealed that clotrimazole pre-incubation decreased E_{max} significantly. Nevertheless, it significantly reduced PIC50 of BK and slightly shifted the DRC to the right. Several mechanisms have been suggested to explain how clotrimazolecauses aortic constriction; the arachidonic acid activates CYT P450 pathway (Metea & Newman, 2006)**.** It has been known that the vasorelaxant effect of EET returns to the opening of BK_{Ca+2} channels and hyperpolarizing of the cell membrane (Juffermans, Kamp, Dijkmans, Visser, & Musters, 2008). CYT P450 enzymes can contribute to arteries and by a production of vasoactive substance rather than NO. CYT P450 enzymes catalyze the formation of EETs, metabolites of arachidonic acid which is putative EDHF that cause relaxation of blood vessels by activating K^+ channels on VSMCs (Roman, 2002).

5. CUNCLUSIONS

Cycloxigenase and epoxygenase inhibitors caused a significant block against relaxation action of BK in aortic rings. Therefore, it can be suggested that BK relaxes aortic smooth muscle partially via the enhancement of these two enzymes. Also, blocking of K_{ir} channel and K_{Ca+2} channels reduced the vasodilators effect of BK, so it can be concluded that BK through opening these channels indirectly perform its relaxant effects.

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