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Differential Kolaviron Attenuated Contractile Responses to Agonists on Isolated Rabbit Aorta in Na^+-K^+ Pump Blockade

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Summary: The mechanism of kolaviron-induced vascular smooth muscles (VSMs) responses has not been fully characterised. The present study investigated the effect and mode of action of kolaviron a biflavanoid-complex and major component of *Garcinia Kola*-fraction on differential contractile responses to agonists-[phenylephrine (PHE) and histamine (HIST)] on VSMs of rabbit isolated aortic rings in K^+-free physiological salt solution (KFPSS). Cumulative concentration responses to PHE and HIST were examined on 2 mm ring segments of the thoracic aortae which were suspended in 20 ml organ baths containing physiological salt solution (PSS) for measurement of isometric contractions, at 37^0C and pH 7.4. The medium was bubbled with 95% O_2, 5% CO_2, and rings were given an initial load of 1g. Cumulative contractile responses to the agonists were studied in normal PSS (control) and following 30 minutes exposure to K^+-free PSS and/or 800µg/mL kolaviron. Contractile responses were expressed as percentage of 80 mM K^+ contractions in normal PSS. Maximal contractions (E_{max}) induced by PHE and HIST compared with high K^+ contraction in the various preparations were differentially altered ($p<0.05$) following exposure to K^+-free or 800µg/mL kolaviron in both intact (+E) and endothelium denuded (-E) rings. Based on the efficacy (E_{max}) and potency (EC_{50}) values for the dose-response curves of the agonists, it is concluded that enhanced differential contractile responses elicited by agonists in K^+-free PSS were significantly attenuated by kolaviron concentration-dependently. This observation probably suggests the existence of another pathway of kolaviron mode of action in vascular smooth muscle reactivity.

Keywords*:* Attenuated contraction, kolaviron, phenylephrine and histamine, Na^+-K^+ pump

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INTRODUCTION

The role of ion channels has been most obvious in the electrically excitable cells; however, the use of ion channel modulators as drugs was operative long before their existence became known and their molecular structures and functions identified (Camerino *et al.*, 2007). The Na^+-K^+ATPase is the plasmalemma pump that generates transmembrane gradients for Na^+ and K^+ that drives several physiological processes including maintenance of the resting membrane potential of cells which is essential in muscle contractions, many transport and exchangers such as the sodium glucose cotransporter, Na^+/Ca^{2+} exchanger, amino acid and vitamin transport into cells and also produces the Na+ gradient that is critical for the reabsorption of Na+ and water, thus maintaining ionic and cellular osmotic homeostasis (O'donnell and Owen, 1994; Lingrel, 2010 and Ofoh *et al.*, 2014). By virtue of its electrogenic nature, the Na^+-K^+ pump also contributes directly to the membrane potential (E_m) and vascular tone. However, inhibition of the Na^+-K^+ ATPase pump by cardiotonic steroids and K^+- free exposure are known to increase contraction and serves as a functional indicator of Na^+-K^+-ATPase activity (Webb and Bohr, 1978; Ko *et al.*, 2008). Kolaviron (KV), a biflavanoid-complex and major component of *Garcinia Kola*-fraction has been reported to exhibit a considerable relaxant effects on intestinal and vascular smooth muscles and its mode of action has been implicated in Na^+-K^+ ATPase activity (Adaramoye and Medeiros, 2009; Udia et al., 2009); although the mechanism of kolaviron vascular effects has not been clearly established. Mechanism of action of vasoactive agents forms one of the principal investigations in vascular reactivity. The present study further investigated the effect and mechanisms of action of kolaviron on differential contractile responses to α_1-adrenergic receptor and H_1-histaminergic receptor subtypes agonists following Na^+-K^+ pump inhibition to establish the role of this enzyme in the modality of action of kolaviron in vascular reactivity on isolated rabbit aortic rings.

MATERIALS AND METHODS

Tissue Preparation:

Ring segments of aorta were obtained from matured New Zealand male and female rabbits which had been

freshly sacrificed throughout the experiment. All experimental procedures complied with the standard protocols for the use of laboratory animals (National Institute of Health USA, 2002). The rabbits were sacrificed by cervical dislocation. The thoracic aorta was carefully dissected out and quickly removed, placed in Petri dish containing physiological salt solution (PSS) and freed of connective tissues, cut into 2-5 mm ring segments and suspended between two L-shaped fine stainless-steel loops in 20 ml jacketed organ baths containing PSS of the following composition (mM/L): NaCl 119.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 24.9, $CaCl_2$ 1.6, and glucose 11.5. The medium was bubbled continuously with 95% O_2 and 5% CO_2 gas mixture and maintained thermostatically at 37°C and pH7.4. Isometric contractions were recorded with force displacement transducers FT.03 connected to Grass model 7D polygraph (Grass instrument Co, Quincy, MA, USA) under an initial load of 1g. An equilibration period of 90 minutes was allowed; following this, aortic rings were stimulated twice with 8 x 10^{-2}M K^+ PSS, at 20-minute interval. The average of these contractions represented the maximum (100%) agonist/KCl which subsequent contractions to agonists [phenylephrine (PHE) or histamine (HIST)] were evaluated.

Protocols.

Concentration-response tests to agonists:

Cumulative concentration-response tests ($1x10^{-9}$ to 2.5 $x10^{-4}$) to each of the two (2) agonists PHE and HIST were examined in normal PSS (control) (n = 8). Concentration-response curves were constructed from which EC_{70} M PHE and HIST were graphically determined.

Kolaviron exposure and contractile-response to agonists in k^+-free PSS:

Cumulative concentration-response tests ($1x10^{-9}$ to 2.5 $x10^{-4}$) to each of the 2 agonists- PHE and HIST were examined in normal PSS (control) (n = 8); as well as following 30 minutes exposure to k^+-*free* (KCl exclusion in normal PSS) and/or 800µg/mL kolaviron 15 minutes into ring exposure (n = 6).

Role of endothelium: In another experiment, the role of vascular endothelium in vascular reactivity was studied in intact (+E) and endothelium denuded (-E) PHE and /or HIST precontracted aortic rings following 30 minutes exposure to k^+-*free* and/or 15 minutes 800µg/mL kolaviron solution (n = 6) to further elucidate the mode of action. Endothelium removal was effected mechanically (Furchgott and Zawadzki, 1980) by gently rubbing the inner lining surface of the rings with a pair of forceps (Ebeigbe *et al.*, 1990). The effectiveness of de-endothelisation was confirmed by lack of relaxation response to 10^{-5}M Acetylcholine (ACh) and more than 70% relation in phenylephrine - precontracted endothelium-denuded and intact arterial rings respectively (Ebeigbe *et al.*, 1990; Olele et al., 1998).

Drugs and solution

K^+-free PSS was prepared by equimolar replacement of K+ with Na+. Drugs used were: Acetylcholine Hydrochloride (Sigma, UK), histamine hydrochloride (Sigma USA), L-phenylephrine hydrochloride (Sigma USA), Tween-80(Sigma, UK). Chemicals used for PSS are: calcium chloride, glucose, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate and sodium chloride (Sigma Chemical Co; Saint Louis, MO, USA). The solutions were prepared fresh on the day of experiments.

Statistical analysis

Data are presented as means ± SEM. Graphs and statistical analyses were by means of OriginPro 8.0 software and Students t-test. P-values less than 0.05 ($p<0.05$) was considered significant; while n-values denote number of animals from which blood vessels were obtained. EC_{70} (concentration producing 70% of maximal contraction) values were derived graphically.

RESULTS

Kolaviron exposure and contractile response to agonists:

Cumulative concentration-response tests (1x 10-9 to 2.5 x10-4) to each of the two agonists- PHE and HIST were examined in normal PSS (control) (n = 8); as well as following 30 minutes exposure to K+-free PSS (n = 8). In figures 1A and 1B, there was a rightward shift of the curve in KV-incubated rings to K+-free response curve suggesting attenuation in agonists cumulative contractile responses.

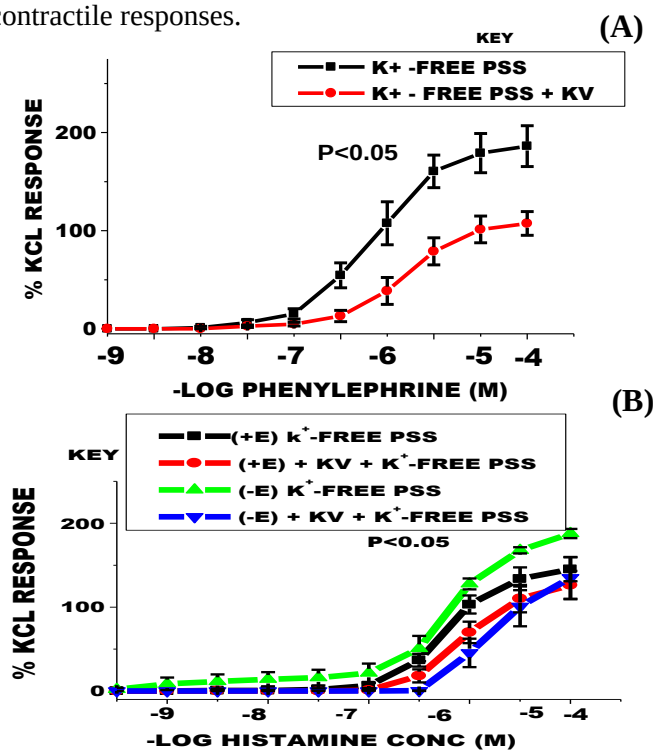

Figure 1: Cumulative concentration response of aorta ring to (A) Phenylephrine and (B) Histamine in the presence or absence of kolaviron, n=6

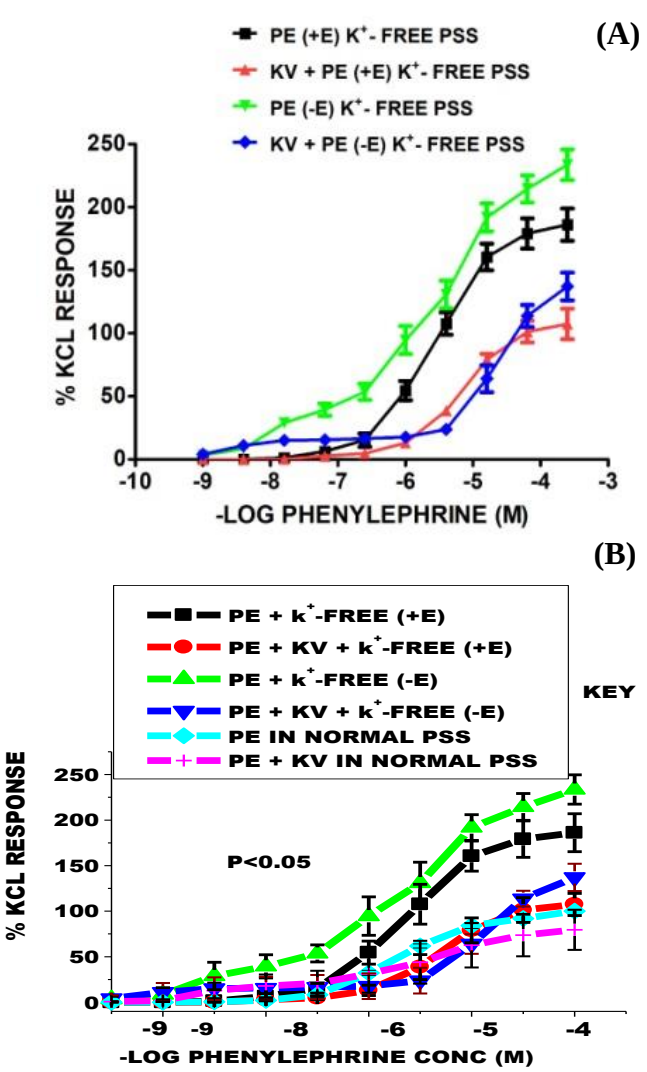

Figure 2: Cumulative contractile response to phenylephrine in (A) intact and (B) endothelium denuded rings incubated with/without Kolaviron. n=6

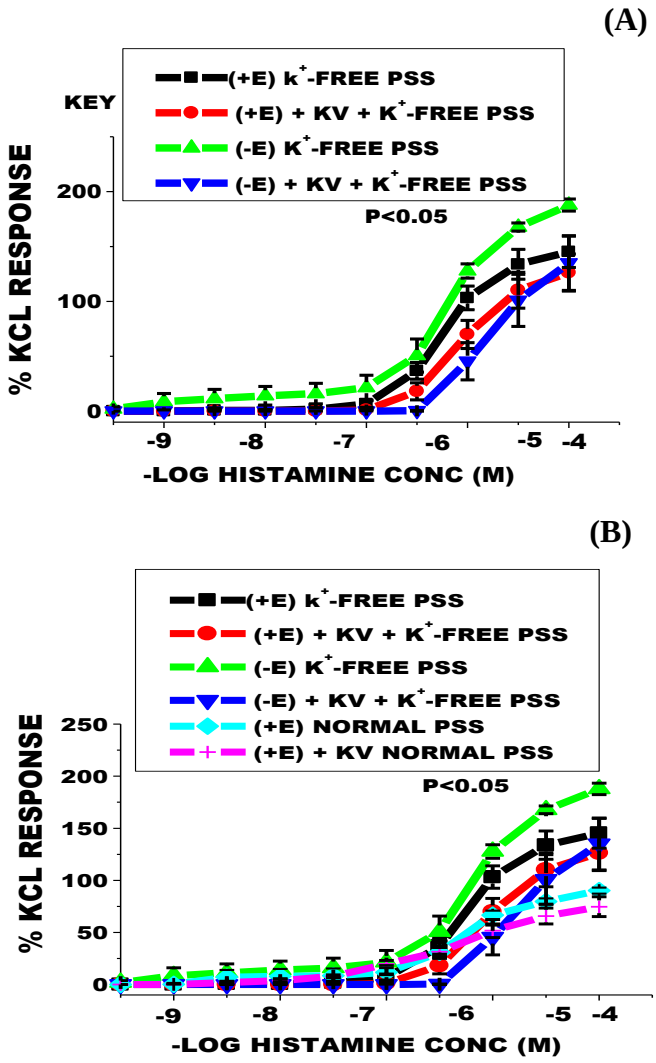

Figure 3: Cumulative contractile response to histamine in (A) intact and (B) endothelium denuded aortic rings incubated with/without Kolaviron, n=6

Role of endothelium in KV-induced attenuated responses

In figures 2A and 2B, exposures to K+-free PSS significantly shifted the cumulative contractile response curves to phenylephrine to the left of the control curve in normal PSS; in both intact (+E) and endothelium denuded (-E) rings, whereas incubation with kolaviron in K+-free PSS significantly ($P<0.05$) shifted the contractile response curves to the right suggesting attenuated contractions; n = 6 , mean ± SEM. Enhancement in contractile responses were however, greater in -E than in +E rings. Similarly, kolaviron-induced attenuated contraction was significantly greater in -E than in +E rings respectively. In figures 3A and 3B, cumulative contractile responses to HIST in K^+- free exposures and/or in KV incubation in both +E and endothelium -E rings followed the same tenet observed in PHE-induced contractions by leftward shift of the control curve in normal PSS. However, based on E_{max} and EC_{50}, enhanced contractile responses and attenuation by KV was greater in PHE than in HIST-mediated responses (n = 6) comparatively. EC_{50}, (PHE: 6.40 ± 0.26 x 10^{-6}, 3.27 ± 0.17 x 10^{-6} 6.00 ± 0.08 x 10^{-6}, 4.25 ± 0.50 x 10^{-6}; HIST: 6.25 ± 0.40 x 10^{-6}, 2.28± 0.50 x 10^{-6}, 6.67 ± 0.40 x 10^{-6}, 3.93 ± 0.70 x 10^{-6})

DISCUSSION

Effects and mechanism of kolaviron (KV) on phenylephrine and histamine-induced cumulative contractile responses in both intact (+E) and endothelium denuded (-E) aortic rings have been studied in vitro and compared in normal physiological solution and in K^+- free exposures. The data presented from these studies have clearly demonstrated that K^+-free exposure to rabbit aortic vascular smooth muscle increases contraction differentially by the two agonists. The tenet was however different in kolaviron-incubated rings in K^+- free exposures which produced a rightward shift in the response curves of the two agonists studied suggesting attenuation in contraction. Phenylephrine-induced contractile responses in K^+- free exposure resulted in the leftward

shift of the response curves concentration-dependently in both intact (+E) and endothelium denuded (-E) aortic rings compared to the response in normal PSS (control); although the enhancement and attenuation in contraction was greater in PHE than in HIST-induced responses. Similarly, histamine-induced cumulative contractile responses in K^+- free exposures followed the same pattern of responses observed in phenylephrine with significant effects only in high doses. This observation is in line with previous reports of increased VSM contraction in Na+-K+ pump inhibition (Webb and Bohr, 1978; Ko *et al.*, 2008). On the contrary, exposure to kolaviron incubated rings in K^+- free PSS produced differential attenuated contractile responses dose-dependently in a rightward shift of the curves of the two agonists in K^+- free PSS (figures 2A and 2B). However, based on the maximal contractions (E_{max}) and EC_{50}-values of the contractile responses of the two agonists, Phenylephrine-induced contractile responses and attenuation in contraction by kolaviron was significantly greater than histamine-induced contractions and attenuation in both -E and +E aortic rings respectively (figures 3A and 3B).

Phenylephrine and histamine are agonists that mediate vascular smooth muscle contraction differentially by activation of α_1-adrenergic receptors (Bolton, 1979 and Vanhoutte, 1984) and H_1-histaminergic receptor subtype (Parsons and Ganellin, 2006) respectively. The greater kolaviron attenuation effect observed in PHE-induced contractile responses in denuded than in endothelium intact arterial rings compared to HIST even though the maximal tensions developed by arterial rings were relatively higher in denuded than intact endothelial rings suggests increased responsiveness of Kolaviron mode of action to α_1-adrenergic receptor and Na/K+ ATPase-depended cellular related activity.

The observation that kolaviron caused attenuated agonists-induced contractions in both +E and - E rings in K^+- free exposure to vascular smooth muscle, is in tandem with earlier observation by Adaramoye and Medeiros (2009) of kolaviron mode of induced relaxant effect on mesenteric vascular smooth muscles of wistar rats and our recent reports that the mechanism of kolaviron-induced relaxation in vascular smooth muscles of rabbit aortic and carotid arterial rings is non-endothelium specific (Uche *et al.*, 2014).

The Na^+-K^+ pump of vascular smooth muscle cells can be stimulated by treatments that increase intracellular Na^+ concentration and/or decrease in intracellular K^+ concentration thereby affecting vascular reactivity (O'donnell and Owen, 1994). Raising the extracellular concentration of potassium ions (K^+) has been demonstrated to inhibit the activity of the endothelial hyperpolarizing factor (EDHF) (Oloyo *et al.*, 2012). Similarly, the function of Na^+-K^+ pump is often regulated by vasoactive hormones, neurotransmitters and growth factors (Lingrel, 2010). Therefore, the role of kolaviron in attenuating PHE and HIST-induced responses in K^+- free PSS exposure may not be unconnected with the existence of a different pathway for its action on vascular smooth muscles. Furthermore, the novel finding of a signalling role for the Na, K+-ATPase provides a completely new dimension for further investigation in the function of this enzyme in vascular smooth muscle reactivity

In conclusion, the role of kolaviron in attenuating phenylephrine and histamine-induced contractile responses in K^+- free PSS exposure is non-endothelium specific and may suggests the existence of other pathway of mode of action of kolaviron in vascular reactivity other than interference with soluble guanylate cyclase enzyme as well as Na^+-K^+ ATPase and /or Na^+-Ca^{2+} exchanger activity.

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