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Research Article

Sickle erythrocytes enhance phenylephrine and histamine contractions of isolated rabbit carotid arteries

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ABSTRACT

The mechanisms of altered vascular reactivity induced by sickle erythrocyte-endothelium interaction remain unclear. The goal of the present study was to examine, comparatively, the influence of sickle erythrocyte on contractile responses induced by phenylephrine and histamine. Concentration-dependent contractile responses were examined in control rabbit carotid artery rings as well as in rings exposed for 30 min to various erythrocyte components obtained from subjects of different haemoglobin (Hb) genotypes (AA, AS and SS), under standard organ bath conditions, as described previously: 2 mm arterial rings preparations were placed in 20ml organ baths containing physiological salt solution (PSS) bubbled with 95% O_2, 5% CO_2, at 37^{o}C and pH 7.4 and isometric contractions measured, under an initial load of 2g. Arterial rings were equilibrated for 60 minutes and then exposed to 50µl of each of the erythrocyte constituents at an adjusted haematocrit of 0.6. The respective EC_{50} (M) values for phenylephrine (PE) and histamine (H) contractions in control carotid arterial rings were 5.1 (±1.4) $x10^{-6}$ (n=7) and 6.3± (1.7) $x10^{-5}$ (n=11). PE contractions were uninfluenced by Hb SS RBCs but significantly enhanced by RBCs from Hb AA and AS subjects: EC_{50} (M) = 7.3 (±6.6) $x10^{-7}$, n=6 and 2.5 (±2.3) x 10^{-6}, n=6 respectively. H contractions were significantly enhanced by only RBCs from Hb AS and SS subjects: EC_{50} values for H is 4.1 (±2.0) x 10^{-5}, n=6 and 4.6 (± 2.1) x 10^{-5}, n=7 respectively. The EC_{50} ratios for PE contractions following exposure to erythrocytes from Hb AA and AS subjects (6.94 and 2.032) respectively are greater than for H contractions following exposure to erythrocytes from Hb AA, AS subjects (0.971, 1.563) respectively, $P<0.05$. These ratios show greater enhancement of PE contractions in the order AA RBC > AS RBC. SS RBC did not significantly alter PE contractions but significantly enhanced H contractions. The greater RBC-induced enhancement of histamine contractions, compared with phenylephrine (in AS and SS), suggests a possible role for histamine in the increased vascular tone and vaso-occlusive crisis in sickle cell disease.

INTRODUCTION

Sickle cell disease is characterized by anaemia, recurring acute vaso-occlusive crises and chronic damage to multiple organs (Hebbel *et al*., 1980). Pathogenesis is due to a single point mutation that results in the substitution of valine for glutamic acid at the sixth position of the β-chain of the hemoglobin S (HBS) molecule (Kaul and Hebbel, 2000). Interaction between sickle erythrocytes and vascular endothelium alters vascular smooth muscle tone (Mosseri et al., 1993; Ajayi et al., 2014) and blood pressure. In our recent study (Ajayi et al., 2013), we reported lower blood pressure values in sickle cell anaemia subjects as well as higher diastolic pressure values in subjects with Hb genotype AS and SS during crisis compared with Hb genotype AA subjects.

Regulation of vascular smooth muscle contraction by vascular endothelial cells is achieved by a balance between vasoconstrictors (e.g. Endothelin-1) and vasodilators (NO). Enhanced production of endothelin-1 and depressed release of endothelium-derived NO, mediate the increased vascular smooth muscle

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contraction during acute vaso-occlusive crises (Mosseri et al, 1993; Olmos et al, 2002; Ergul et al, 2004).

Various other workers have reported that endothelial dysfunction occurs in sickle cell disease may be result from increased wall shear stress, adhesion and interaction between sickle red cells and endothelial cells, low oxygen tension as well as increased viscosity (Quyyumi et al.,1997). Stuart et al. (1999) suggested that the enhanced interaction between sickle erythrocytes and the vascular endothelium may contribute to the occurrence of vaso-occlusive crisis. Also, intravascular hemolysis has been reported to impair nitric oxide (NO) bioavailability which may result in diminished blood flow, regional vasoconstriction and subsequent blood vessel remodelling (Kato et al, 2017). Endothelial nitric oxide-dependent acetylcholine-induced relaxation is attenuated by sickle erythrocytes (Mosseri et al., 1993); however, the mechanisms of altered contractile responses by vasoactive agents induced by sickle erythrocyte-endothelium interaction remain unclear. The goal of the present study was to examine, comparatively, the influence of sickle erythrocytes on contractile responses induced by phenylephrine and histamine in isolated rabbit carotid arteries.

METHODS

Samples

Blood samples were obtained from patients of different Hb genotypes, attending the University of Benin Teaching Hospital. Erythrocytes (RBCs) were prepared according to the method of Caughley and Watkins, (1985), by washing with normal saline, to obtain a clear supernatant. The cells were re-suspended to make up 6-8% packed cell volume.

Tissue preparation

Segments of the carotid arteries were obtained from freshly-sacrificed, New Zealand rabbits, cleaned free of adhering connective tissues and cut into 2mm rings. The rings were place between L-shaped wire loops and suspended in 20ml organ baths containing Physiological Salt solution (PSS). The lower loop was attached to the base of the organ bath while the upper end was attached to a Grass model FT03 force transducer connected to a Grass model 7P polygraph (Grass Instrument Co, Quincy, MA, USA). The composition of the PSS was (mM): 119 NaCl, 4.7 KCl, 1.6$CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 24.9 $NaHCO_3$ and 11.5 glucose. The PSS was bubbled with 95% O_2-5% CO_2 gas mixture. The rings were given an initial load of 2g, at 37^{o}C and pH 7.4 and were allowed to equilibrate for 60 minutes.

Protocol

The rings were first contracted with 80mM K^+, to induce reference maximal (100%) contraction - against which subsequent contractions were compared.

Concentration-response to phenylephrine (PE) and histamine (HIST) were examined by cumulative addition of the agonists to the baths in control rings as well as in rings exposed for 20 minutes to RBCs obtained from the three genotype groups (AA, AS, SS).

Statistical analysis

Results are presented as means ± SEM. Comparison of the means was done using student's *t*-test and the MicroCal Origin 5.0 software. A p value < 0.05 was considered statistically significant. EC_{50} (M) values (concentrations producing 50% max. responses) were determined graphically. EC_{50} ratio represents EC_{50} values in the absence : presence of agonist.

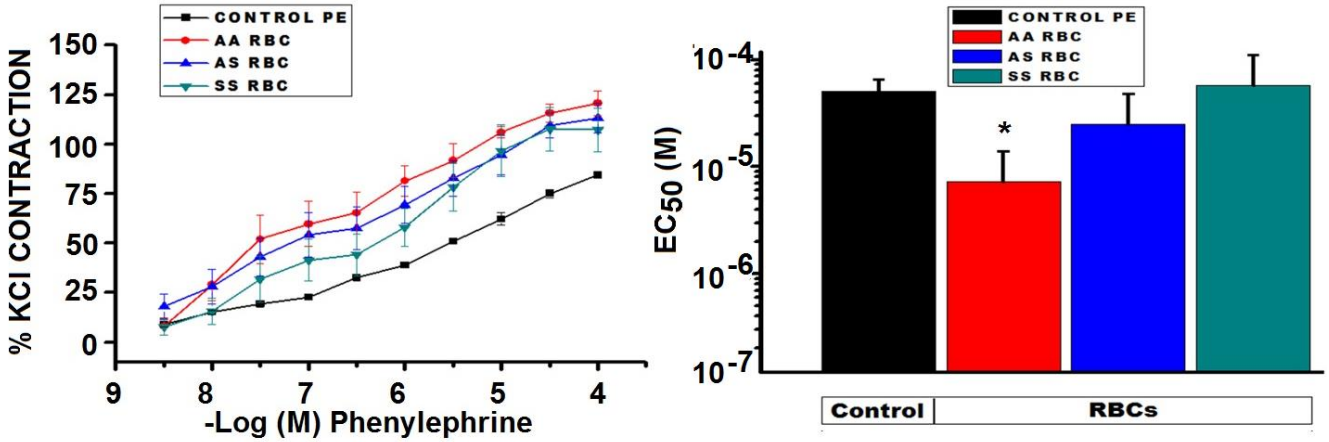

Fig. 1. Dose-response curves for phenylephrine (PE) contractions in control rings as well as in rings exposed to RBCs from subjects of different haemoglobin genotypes. Means ± SEM; n = 11. *denotes significant difference from control.

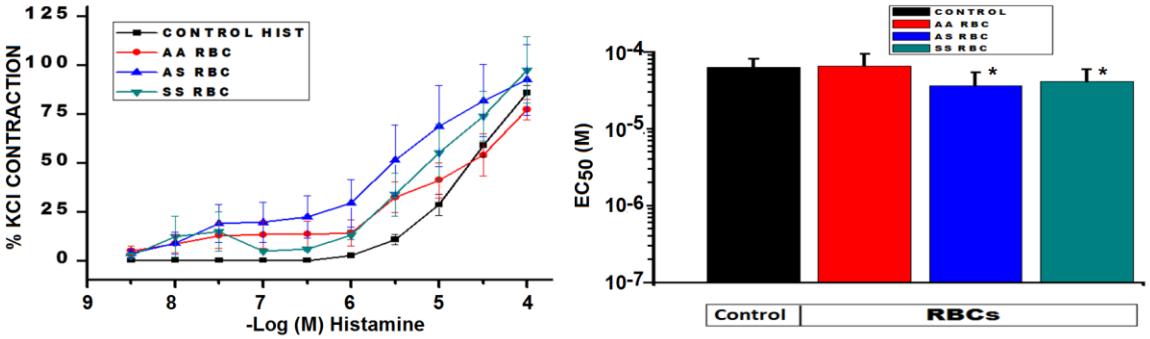

Fig. 2. Dose-response curves for histamine contractions in control rings as well as in rings exposed to RBCs from subjects of different haemoglobin genotypes. Means ± SEM; n = 11. *denotes significant difference from control.

RESULTS

Dose-response to Phenylephrine

The dose-response curves for phenylephrine contractions are shown in Fig. 1. In comparison with the control, phenylephrine contractions were significantly enhanced following exposure to RBCs from the 3 genotype groups.

Dose-response to Histamine

Histamine contractions were significantly enhanced following exposure to RBCs especially from genotypes AS and SS (Fig. 2). The EC_{50} values presented illustrate the moderate, though significant enhancement of histamine contractions.

EC_{50} and EC_{50} ratio values for Phenylephrine and histamine contractions

Based on the EC_{50} values (Fig. 1), phenylephrine contractions were uninfluenced by Hb SS RBCs but significantly enhanced by RBCs from Hb AA and AS subjects. The EC_{50} ratios for phenylephrine contractions following exposure to erythrocytes from Hb AA and AS subjects (6.94 and 2.032) respectively, are significantly greater than for histamine contractions following exposure to erythrocytes from Hb AA, AS subjects (0.971, 1.563) respectively, $P<0.05$.

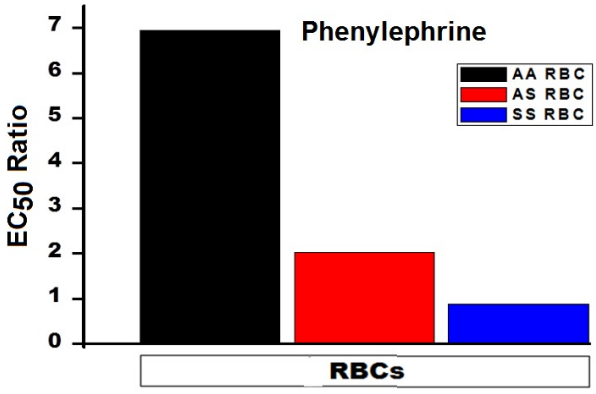

Fig. 3. EC_{50} ratios showing enhancement of phenylephrine contractions following exposure of rings to RBCS from Hb genotype AA subjects

These ratios show greater enhancement of phenylephrine contractions in the order AA RBC > AS RBC. SS RBC did not significantly alter phenylephrine contractions but significantly enhanced histamine contractions.

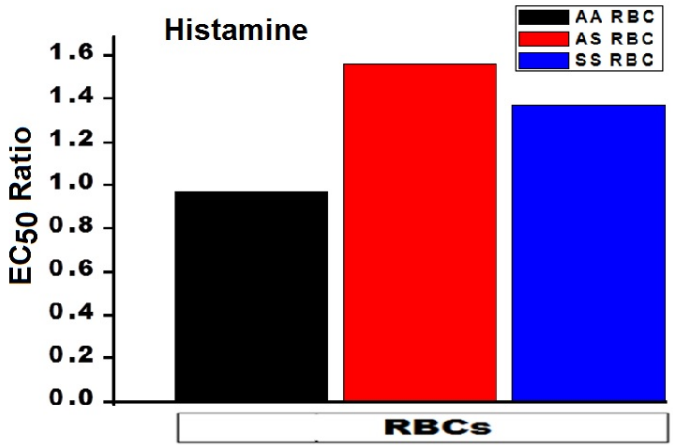

Fig. 4. EC_{50} ratios showing greater enhancement of histamine contractions following exposure of rings to RBCS from Hb genotype AS and SS subjects.

DISCUSSION

We have previously reported (Ajayi and Ebeigbe (2014) as well as others (Mosseri et al, 1993), that constituents of erythrocytes modulate vascular reactivity through interaction with the vascular endothelium. The results of the present study demonstrate that there is a differential enhancement of phenylephrine and histamine contractions of rabbit carotid arteries following exposure to erythrocytes from subjects with different Hb genotypes (AA, AS, SS).

A direct action of sickle erythrocytes on microvessels has been reported to result in vaso-occlusion. Sickle erythrocytes also act indirectly (Weinstein et al., 1990). It has been suggested that the vaso-oclussion which occurs during sickle cell disease crises may be

associated with increased vascular tone/vasospasm (Stuart et al,1999).

The differential effects of RBCs from subjects of different Hb genotypes on phenylephrine and histamine contractions of rabbit carotid arteries suggests that circulating agonists may differentially contribute to vasoocclusion and/or vasospasm. Histamine has been reported to modulate cardiovascular function: A study by Kalsner and Richards, (1984 reported that coronary arteries of some patients with coronary artery disease are hyper-responsive to histamine and contain significantly higher concentrations of histamine.

Thus, the observation, in this study, showing greater RBC-induced enhancement of histamine contractions, compared with phenylephrine (in AS and SS), suggests a possible role for histamine in the increased vascular tone and vaso-occlusive crisis in sickle cell disease.

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