

Review Article

Influence of haemoglobin solution from sickled erythrocytes on endothelium-dependent relaxation of isolated rabbit carotid arteries

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Vasorelaxation, EDRF, sickle cell anaemia, endothelium, haemoglobin solutions, haemoglobin genotypes.

ABSTRACT

There are conflicting reports concerning vascular reactivity changes in sickle cell anaemia (SCA). The goal of the present study was to examine possible interactions between components of sickle erythrocytes and the vascular endothelium, that may alter vascular reactivity in isolated rabbit carotid arteries. Endothelium-dependent acetylcholine (Ach)-induced relaxation responses (following phenylephrine pre-contractions) 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: 2 mm rings suspended in 20ml organ baths containing physiological salt solution (PSS) bubbled with 95% O₂, 5% CO₂, at 37°C and pH 7.4 and isometric contractions measured, under an initial load of 2g. Arterial rings were exposed to 50µl of each of the erythrocyte constituents at an adjusted haematocrit of 0.6. Intact Erythrocytes of Hb AA and SS had no effect on Ach relaxation while AS erythrocytes caused a significant inhibition (P<0.05) by 25%. Exposure to Hb solutions from Hb AA and AS subjects had no inhibitory effect on Ach relaxation whereas a 30% inhibition occurred in Hb SS. Erythrocyte ghosts produced no inhibition with Hb AA, AS and SS. We propose that the haemoglobin content of Hb SS appears likely to be associated with an enhanced inhibition of endothelial function in Hb SS subjects and may predispose more cardiovascular complications especially during haemolytic crisis in SCA.

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INTRODUCTION

Mechanism of vascular relaxation has been inextricably linked with the endothelium and the endothelial derived relaxing factor (EDRF) have been characterized and known as nitric oxide

(Furchgott and Zawadski, 1980, Ebeigbe and Cabanie, 1992; Mosseri et al., 1993; Olmos et al., 2002). We have recently reported significantly lower blood pressures in SCA subjects and demonstrated that diastolic pressure values were significantly higher in Hb AS subjects and Hb SS during crisis than Hb AA (Ajayi et al., 2013). Also, we have characterized *in vitro*, the modulatory role of erythrocyte components in different Hemoglobin (Hb) genotypes on contractile responses induced by phenylephrine (PE) in isolated rabbit carotid arterial smooth muscle (Ajayi and Ebeigbe, 2014).

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Sickle erythrocytes have been reported to have direct vaso-occlusive properties on micro vessels (Kaul et al., 1989) or indirectly alter endothelial functions such as Prostacyclin production and DNA synthesis (Weinstein et al., 1990). Also, anatomical studies have revealed endothelial damage in patients with SCA (Klug et al., 1982), observed that the potentials for altered endothelial regulation of vaso-reactivity in the pathogenesis of vaso-occlusive events in SCA remains unexplored. Furthermore, Nitric oxide (NO) levels has been reported to be significantly lower in SCA patients in both stable and crisis states (Lopez et al., 2000), but the mechanism involved is still not clear. These conflicting reports of altered vascular reactivity in sickle cell anaemia (SCA) have prompted us, in this study, to examine possible interactions between sickle erythrocytes (and RBC components) and the vascular endothelium, that may likely influence vascular reactivity and to characterize the roles of genetic inheritance of the A and S genes play in modulating vascular relaxation.

MATERIALS AND METHODS

Rabbits were freshly sacrificed by stunning dislocation of the neck and carotid arteries isolated. Segments of the carotid arteries were obtained, 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, 1.6CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 24.9 NaHCO₃ and 11.5 glucose. The PSS was bubbled throughout with 95% O₂-5% CO₂ gas mixture. The rings were given an initial load of 2g, at 37°C and pH 7.4. They were allowed an equilibration time of 90 minutes before the commencement of various protocols. The rings were first contracted by EC₇₀M PE (previously determined) followed by relaxation responses by EC_{max} Ach, mean values

were taken as 100%. Further relaxation responses following the experimental protocol with different erythrocyte components were recorded and expressed as a percentage of the reference relaxation induced by Ach. The protocol is as follows: Relaxation responses to EC_{max}M Ach in arterial rings exposed to various erythrocyte components obtained from subjects of different Hb genotypes (AA, AS and SS) were examined in control rings as well as in rings exposed for 30 minutes to (a) intact washed erythrocytes (b) erythrocyte ghosts and (c) haemoglobin solution. Arterial rings were exposed to 50µl of each of the erythrocyte constituents at an adjusted haematocrit of 0.6. In order to examine the impact of each component on the Ach-induced relaxation responses, the percentage reduction in relaxation was calculated by subtracting the magnitude of relaxation after exposure of different components from that of control, and dividing the value with that of the control multiplied by 100%.

Statistical analysis

Results are presented as means ± SEM and comparison of the means was done using student's *t*-tests. A *p* value < 0.05 was considered as statistically significant. Relaxation responses were expressed as percentage (%) of maximal response to EC₇₀M Ach. The dose-response curves for Ach were constructed using a computer software Origin™5.0 (Microcal Software Inc, Northampton, USA) and EC₅₀, EC₇₀ values (concentrations producing 50% and 70% max. responses) were determined graphically.

RESULTS

The data presented in Figs 1-4 show the effects of various components of erythrocytes obtained from subjects with different haemoglobin genotypes on the relaxation responses induced by acetylcholine in isolated rabbit carotid arteries.

The arterial rings were exposed for 30 minutes to intact washed erythrocytes, erythrocyte ghosts and haemoglobin solution.

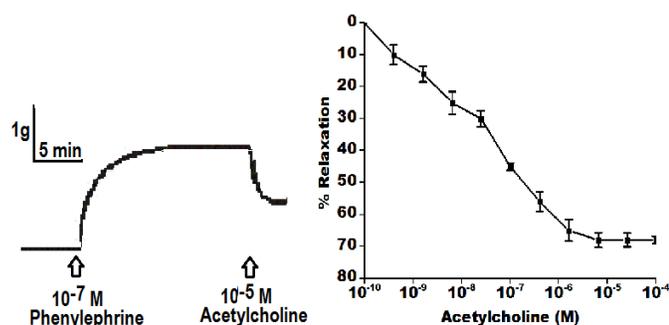


Fig. 1. Typical tracing (left) showing relaxation response induced by a single dose of Ach following PE precontraction. In test experiments, rings were exposed to erythrocyte components prior to and during PE contraction and subsequent Ach relaxation. Graph (right) shows Acetylcholine induced relaxation response in pre-contracted rings of rabbit carotid arteries. Values are expressed as percentage pre-contraction induced by EC₇₀ (1.6 x 10⁻⁶M) phenylephrine. Data are means ±SEM (n=14).

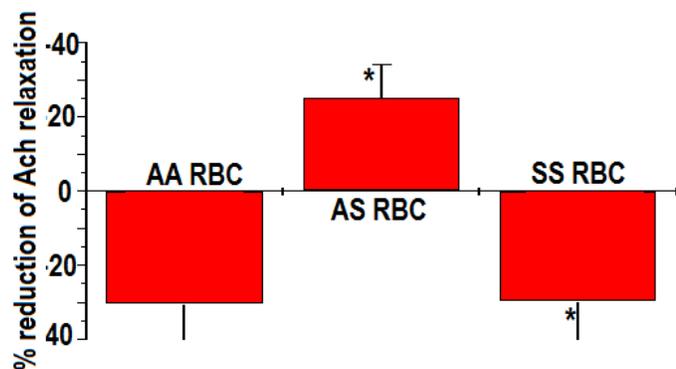


Fig. 2. % Reduction of Ach-induced relaxation following exposure to RBCs from AA, AS and SS subjects. There was a statistically significant (*) increase of inhibition of Ach relaxation with Hb AS RBC (P<0.05), while HB SS RBC shows a significantly enhanced (*) Ach-induced relaxation (P<0.05).

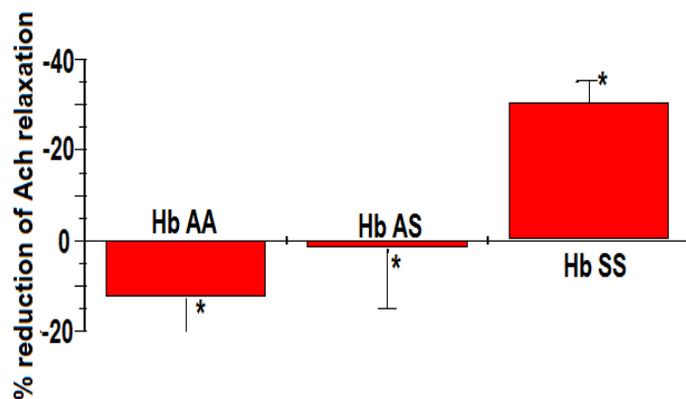


Fig. 3. Histogram showing the reduction of Ach relaxation following interaction between Hb solution and the endothelium. Ach-induced relaxation responses occurred with Hb solutions from Hb AA and Hb AS but not with Hb SS subjects. (P<0.05, respectively), while haemoglobin solution from Hb SS shows a significant inhibition of Ach-induced relaxation (P<0.05).

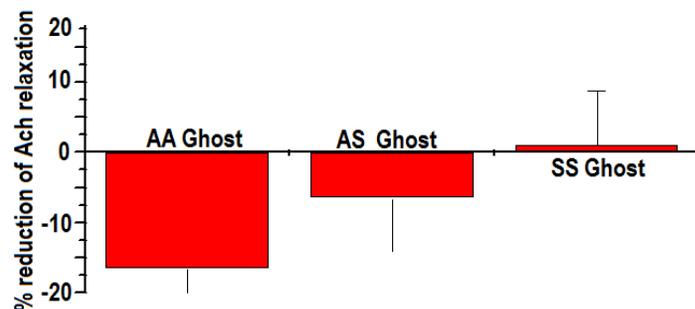


Fig. 4. Histogram showing interaction between ghosts from subjects with different Hb genotypes and the vascular endothelium. There were no significant differences in the Ach-induced relaxation responses after exposure to ghosts from the different Hb genotypes (p>0.05, respectively).

DISCUSSION

Acetylcholine (Ach) induces vasorelaxation by stimulating the synthesis and release of endothelium-derived relaxing factor (EDRF), which has been identified as nitric oxide (NO), (Furchgott and Zawadzki, 1980, Ebeigbe and Cabanie, 1991; Mosseri et al., 1993; Olmos et al., 2002). The effect of the haemoglobin as a scavenger of nitric oxide has been described (Ebeigbe et al., 1994; Moncada and Higgs, 1993; Ajayi et al., 2000; Olmos et al., 2002). Also, Olmos et al., (2002) reported a reduced ability of sickle cells to scavenge EDRF-NO. Haemoglobin inhibits endothelium-dependent relaxation by inactivating EDRF (Martin et al., 2005).

In this study, we have observed significantly inhibition (by 25%, $P < 0.05$), relaxation responses to acetylcholine (Ach) following exposure to intact erythrocytes obtained from Hb AS genotype subjects, in contrast with intact erythrocytes from Hb AA and Hb SS subjects, which did not significantly alter Ach-induced vasorelaxation. This observation is consistent with the reports of Olmos et al., (2002) who showed that the ability of sickle cells to scavenge endothelium derived NO is reduced.

The solutions of haemoglobin prepared from the Hb SS subjects, produced significant inhibition relaxation response to Ach (by 30%), in contrast with Hb solution from Hb AA and Hb AS subjects which did not significantly alter the relaxation responses. This contradicts the reports of Olmos et al., (2002), where they observed that haemoglobin A and S have comparable effects on vasorelaxation in an oxygenated physiological solution (PSS) since they both have equal affinity for oxygen in solution and oxyhaemoglobin is the most potent NO scavenging form of haemoglobin (Moncada and Higgs, 1993). However, this study did not show the same effect with AA subjects; although there was no inhibition of relaxation with Hb AS, the magnitude of relaxation appears to show genetic trait. Exposure of arterial rings to erythrocyte Ghosts obtained from all the subjects (Hb genotypes AA, AS and SS) did not produce any significant inhibition of Ach-induced relaxation; although Hb SS exhibited a 2% inhibition which seems not sufficient in magnitude to reflect any significant effect, it is plausible to speculate that the combined effect with haemoglobin may be synergistic, resulting in greater alteration of Ach-induced relaxation especially during haemolysis.

The previous reports of low levels of NO in SCA patients by Lopez et al., (2000), and that of Olmos et al., (2002) that Sickle cells have reduced ability to scavenge NO appears conflicting; the present study proffer feasible explanations to the two views: while intact sickle cells have reduced ability to scavenge NO on one hand (stable state), their haemoglobin have the greatest ability to inhibit Ach-induced endothelial relaxation of the other hand (during haemolytic episode).

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