Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences
http://www.jaaps.aapsnet.org

Research Article

Enhanced Phenylephrine Contractions in Rabbit Carotid Arteries Following Exposure to Haemoglobin from Subjects with Sickle Cell Trait.

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Keywords:	ABSTRACT
Phenylephrine, *Sickle cell trait,* *Haemoglobin solution,* *Erythrocytes*	We have previously reported raised resting diastolic blood pressure (BP) in subjects with single S-gene inheritance – although the mechanism was unclear. The goal of this study was to characterize, in vitro, the modulatory role of erythrocyte components from subjects with different Hemoglobin (Hb) genotypes on contractile responses induced by phenylephrine (PE) in isolated rabbit carotid arterial smooth muscle. Carotid arteries were isolated from rabbits and cut into 2mm rings, suspended in 20ml organ baths and bubbled with 95% O_2, 5% CO_2 and isometric contractions examined under an initial load of 1g, at 37°C and pH 7.4. Contractile responses to EC_{70} (M) PE 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. The magnitudes of the PE-induced contractions with intact erythrocytes were: 1180±202, 1700±260 and 900±302 for Hb AA, Hb AS and Hb SS respectively (n=13); these values were significantly increased following exposure to various erythrocyte components in the order: RBC> HB > Ghost (P<0.05, respectively). There were no significant differences in PE contractions following exposure to intact erythrocytes and ghosts from subjects with different Hb genotypes; however, exposure to haemoglobin solution significantly enhanced PE contractions in Hb AS subjects than in Hb AA and Hb SS (P<0.05 respectively). In conclusion, we reason that the haemoglobin content of Hb AS erythrocytes may be responsible for the enhanced contractile responses to PE and may explain in part, why diastolic BP values remain high in Hb AS subjects.

INTRODUCTION

Blood pressure regulation requires that organ perfusion be achieved through a very dynamic range of physiologic activities and environments. Genetic approaches have advanced the knowledge of biological pathways underlying inter-individual variations in blood pressure and cardiovascular diseases. Increased diastolic blood pressure in heterozygote Hb AS subjects than Hb AA and Hb SS has been earlier reported by Reid and Anah, (1985) and most recently reiterated by Ajayi *et al*., (2013), with a plausible question of the exact mechanism by which the single S-gene mediate the vascular interactions resulting in this observation. The role of vascular endothelium is significant in the regulation of blood pressure. Endothelial cells synthesize vasodilatory factors like nitric oxide (NO), (Berthe et al., 2011). Arginine is the rate limiting

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substrate for endothelial nitric oxide synthase (eNOS), which acts as a catalyst in NO production (Visigalli et al, 2010). Phenylephrine is a selective α_1-adrenergic agonist-mediated force generator in vascular smooth muscle contraction. Vasoconstriction of arterioles and resistance arteries induced by α_1-adrenergic agonists is associated not only with an increase in the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_{in}$) in vascular smooth muscle cells (VSMCs) but also with an increase in $[Ca^{2+}]_{in}$ in the endothelial cells (ECs) lining these vessels (Dora et al., 1997; Schuster et al., 2001). The modulatory role of different components of erythrocytes especially the haemoglobin in vascular smooth contraction has to the best of our knowledge not been reported. Our aim therefore is to highlight the effects of exposure of smooth muscle rings to intact erythrocyte from different haemoglobin genotypes as well as interactions with different components of the red cell (haemoglobin solution and ghosts).

MATERIALS AND METHODS

Rabbits were 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, 1.6CaCl$_2$, 1.2 MgSO$_4$, 1.2 KH$_2$PO$_4$, 24.9 NaHCO$_3$ and 11.5 glucose. The PSS was bubbled throughout with 95% O$_2$-5% CO$_2$ gas mixture. The rings were given an initial load of 2g, at 37°C and pH 7.4. they were given an equilibration time of 90 minutes before the commencement of various protocols. The rings were first contracted by 80mM K+ and this response was taken as 100%. Contractile responses were each expressed as a percentage of the contraction previously induced by 80mMKCl. Dose-response to phenylephrine was carried out by cumulative addition of the agonist to the bath. Contractile responses to EC$_{70}$M PE 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.

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. Contractile responses are expressed as percentage (%) of maximal response to 80mM KCl. The dose-response curves for PE were constructed using a computer software Origin™5.0 (Microcal Software Inc, Northampton, USA) and EC$_{50}$, EC$_{70}$ values (concentrations producing 50% and 70% max. responses) were determined graphically.

RESULTS

Contractile Responses To Phenylephrine
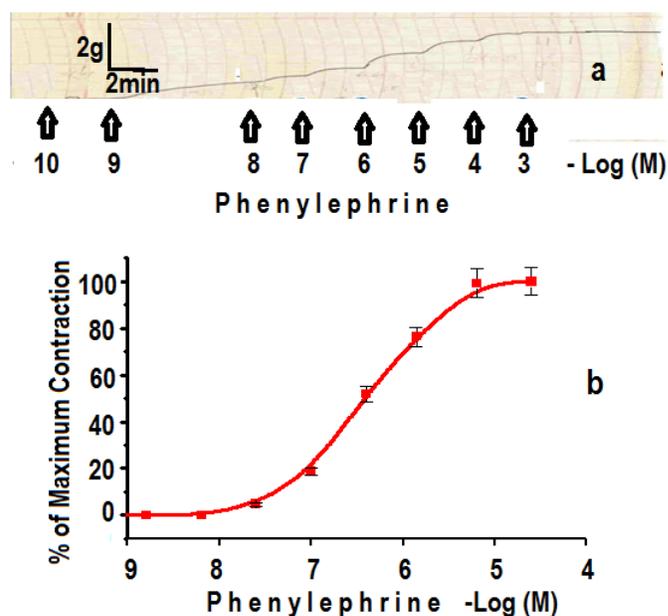Phenylephrine elicited concentration-dependent contractions in all experiments (Fig. 1).

Fig. 1
Typical tracing (a) of cumulative phenylephrine contractions in a rabbit carotid artery ring. Responses in (a) are summarized in (b). Arrows indicate points of drug administration Values denote means ± SEM; n = 14.

Exposure to RBC components
Exposure to different erythrocyte components resulted in varying alterations of Phenylephrine contractions.
The magnitude of the PE-induced contractile responses was significantly increased following exposure to various erythrocyte components in the order RBC> HB > Ghost (P<0.05 respectively). This indicated that the interactions of the artery with intact erythrocytes and

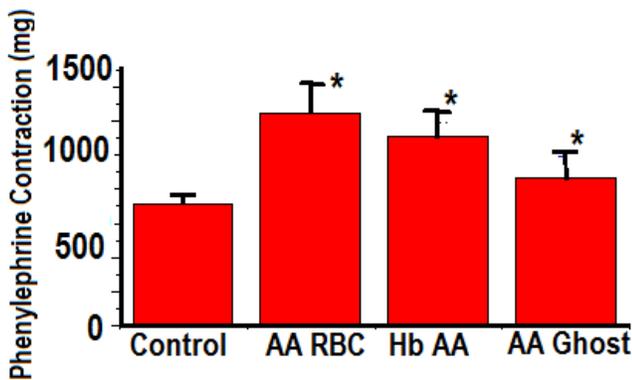haemoglobin significantly potentiate the PE contractile responses but not with the Ghost.

Fig. 2
$10^{-7}M$ (EC_{70}) Phenylephrine-induced contractions in control and following exposure to various components of RBC obtained from subjects with Hb AA genotype.***Denotes** significant difference from control

There were no significant differences in the magnitudes of PE contractions. The values were compared against each other (P>0.05 respectively).
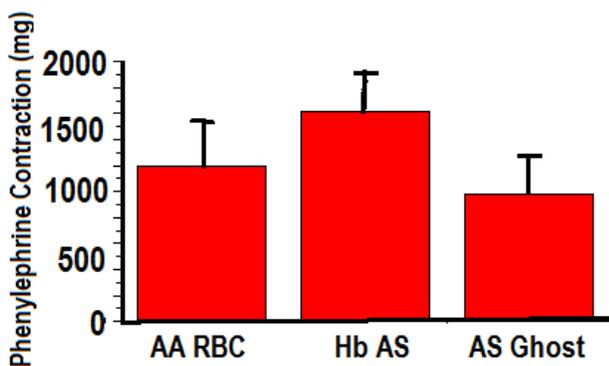

Fig. 3
Comparisons of $10^{-7}M$ phenylephrine pre-contractions in rabbit carotid arteries following exposure to different RBC components from HbAS subjects. Differences are not significant.
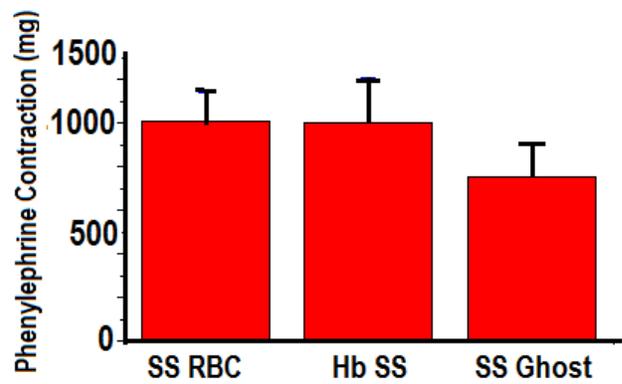

Fig. 4
Comparison of magnitudes of $10^{-7}M$ phenylephrine contractions in rabbit carotid arteries exposed to different RBC components from HbSS subjects. Differences are not significant.

There were no significant differences in the magnitude of PE contractions following exposure to the different RBC components from HbSS subjects (Fig. 4). The values were compared against each other (P>0.05 respectively).
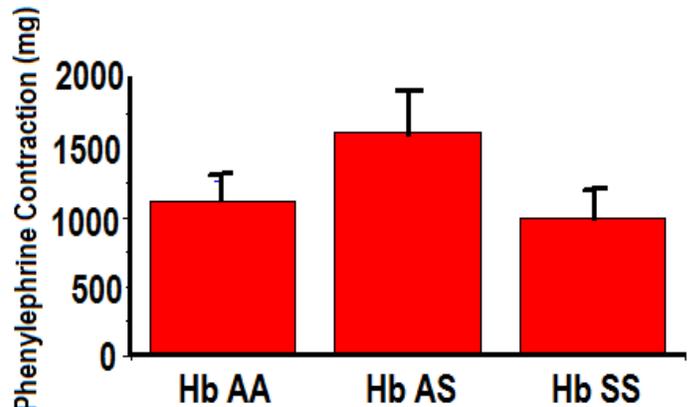

Fig. 5
Comparison of magnitudes of phenylephrine contractions following exposure to haemoglobin solutions from different genotype subjects.

Exposure to Hb from AS subjects resulted in a significant increase in phenylephrine contractions in comparison with Hb AA and HB SS (P<0.05, respectively), while there were no differences between that of Hb AA and Hb SS (Fig. 5).

DISCUSSION

Vascular tone is influenced by a wide variety of contractile agents. In this study, we have used

phenylephrine (PE), an α_1 receptor activator, to induce vascular contractile responses. The rise in the cytosolic calcium ions following stimulation by α_1-adrenergic stimulation depends on the expression of functional α_1-adrenergic receptors in the endothelial cells (Vinet *et al*., 2000; Vinci *et al*., 2007).

Our observations have shown that phenylephrine – induced contractions following exposure to intact erythrocytes from normal (Hb AA) and SCA subjects are not significantly different. On the other hand, PE-induced contractions following exposure to haemoglobin solution were significantly raised, greatest for Hb AS subjects (P<0.05) and in the order: Hb AS> Hb AA > Hb SS. A probable explanation for the greater effect of Hb solution (from Hb AS subjects) on the enhanced PE contractions is the possibility that the effect of the haemoglobin as a scavenger of nitric oxide (Ebeigbe et al., 1994; Moncada and Higgs, 1993; Ajayi et al., 2000) is perhaps greatest in Hb AS subjects compared Hb AA and Hb SS subjects. Also, Olmos et al., (2002) reported a reduced ability of sickle cells to scavenge EDRF-NO. The observation that exposure to erythrocyte Ghosts as to intact erythrocytes did not significantly alter contractile responses to PE (in contrast with the effect of Hb solution) suggests an important role for Hb in modulation of contractile responses to Phenylephrine.

Our earlier observations, therefore, of increased diastolic pressure in Hb AS subjects (Ajayi et al., 2013), as well as increased systolic and Diastolic blood pressures in SCA subjects during crisis could partly be explained in terms of the enhancement of PE -induced contractions by haemoglobin as discussed above. It is pertinent to note some published studies which showed that in vivo interactions between vascular endothelial cells and haemoglobin (under physiologic conditions) results in degeneration of senile erythrocytes and also that Interactions between the endothelium and erythrocytes may contribute to the vascular complications of sickle cell disease (Ibe et al., 1997; Belhassen et al., 2001) . This effect is aggravated during haemolytic crises in SCA patients (Olmos et al., 2002). Though, there is paucity of information linking other haemoglobin genotypes and haemoglobin interactions with such observations, this study therefore, has highlighted the possible roles of haemoglobin molecules in the mechanism of tension generation within the vasculature of SCD subjects especially Hb AS and SS subjects (during crisis).

The exact mechanism involving the population of functional receptors for α_1-adrenergic agonists being up-regulated or the expression of more endothelial cells to release more calcium ions may not be explained by the present study.

The erythrocyte ghosts in all indications have little or no effect on tension generation, therefore, the observed effects by intact erythrocytes could be associated with membrane leakages of haemoglobin especially with Hb AS.

In summary, our data suggest a role for haemoglobin in the enhanced contractile response to PE especially with Hb genotype AS, while the observed increases in diastolic blood pressure in Hb AS as well as SCD during crisis may be explained in part by the interactions between the leaked haemoglobin in the vasculature.

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