J. Afr. Ass. Physiol. Sci. 3 (2): 107 – 109, December, 2015.

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

Research Article

Influence of endothelium on the membrane-stabilizing effect of calcium

S.O. Azubuike-Osu[1]* and A.B. Ebeigbe
Departments of Physiology, [1]Federal University, Ndufu-Alike Ikwo, Abakaliki and [2]University of Benin, Benin City, Nigeria.

Keywords:
Calcium, rabbit aorta, Vascular smooth muscle, Membrane stabilization, Endothelium, methylene blue, Guanylate cyclase

ABSTRACT

Background: A decrease in membrane permeability to calcium ions, caused by increased extracellular calcium concentration is referred to as membrane-stabilization. There is a paucity of information on the role of vascular endothelium in the membrane-stabilizing effect of Ca^{2+} ions. The goal of the present study was to examine the influence of the endothelium on the membrane-stabilizing effect of Ca^{2+} ions in rabbit aortic smooth muscle. **Methods:** Isometric contractions of 2mm ring segments of rabbit aorta, placed in 20ml organ baths containing physiological salt solution (PSS) and bubbled with 95% O_2, 5% CO_2 gas mixture at 37^0C and pH 7.4 were examined. The magnitude of the relaxation responses induced by increasing extracellular Ca^{2+} concentration from 5.0 to 25mM in phenylephrine pre-contracted rings was taken as an indirect indicator of the membrane-stabilizing effect of Ca^{2+}. The relaxation responses induced by 25mM Ca^{2+} were estimated in endothelium-intact, endothelium-denuded rings as well as following exposure to 10^{-6}M methylene blue. **Results:** In all experiments, an increase in $[Ca^{2+}]_o$ (low bicarbonate PSS) from 5.0 to 25.0mM in rings with intact endothelium resulted in relaxation responses. These relaxation responses were attenuated in endothelium-denuded rings as well as following exposure to methylene blue. **Conclusion:** The results show that relaxation responses induced by high Ca^{2+} due to membrane stabilization is endothelium-dependent.

INTRODUCTION

A role for Ca^{2+} ions in endothelium-dependent relaxation responses has been well-reported (Edwards et al.,1985; Fleming et al, 1998; Saunders et al, 2000; Gilles, 2006). Vascular smooth muscle relaxation is mediated by a decreased intracellular Ca^{2+} concentration and increased MLC phosphatase activity (Webb, 2003). Also, Ca^{2+} influx into endothelial cells has been shown to play an important role (Long and stone, 1985; Loeb et al., 1988) in the release and/or synthesis of endothelium-derived vasorelaxant (EDRF). The relaxation response of vascular smooth muscle induced by high concentrations of calcium after pre-contraction by noradrenaline is referred to as the "membrane-stabilizing effect" of calcium (Webb and Bohr, 1978; Ebeigbe and Aloamaka 1987). Early reports suggest that the membrane-stabilizing effect of Ca^{2+} is attenuated by raised extracellular Mg^{2+} (Ebeigbe and Aloamaka, 1987) and the calcium channel blocker, D600 (Webb, 1982). A role for vascular endothelium in Ca^{2+} relaxation of rat aortic smooth muscle has been suggested (Wu and Bohr, 1991). In view of the known species and regional variations in vascular smooth muscle responses to vasoactive agents (Bolton, 1979), the present study was designed to examine the role of vascular endothelium in the relaxation of isolated rabbit aortic smooth muscle induced by high concentration of Ca^{2+} ions.

Address for correspondence:
E-mail: sharonoluchi@yahoo.co.uk
Tel.: +2348050542444

MATERIALS AND METHODS

Tissue Preparation

Male and female Rabbits were freshly sacrificed by stunning and the aorta was isolated. Segments of the aorta were cleaned free of adherent connective tissues and cut into 2mm rings. The cut rings were suspended

between L-shaped wire loops in 20ml organ baths containing Physiological Salt Solution (PSS). The lower loop was attached to the base of the organ bath while the upper loop was attached to a Grass Model FT03 force Transducer connected to a Grass Model 7P Polygraph (from Grass Instrument Co., Quincy, MA, USA). The composition of the normal PSS was (mM): 119 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.2 $CaCl_2$, 24.9 $NaHCO_3$, 11.5 $C_6H_{12}O_6$, 0.03 $CaNa_2EDTA$. In studies on the membrane-stabilizing effect of calcium, a low-bicarbonate PSS (pH 7.2) containing (mM): 150 NaCl, 5.4 KCl, 1.2 KH2PO4, 1.2 MgSO4, 6.0 NaHCO3, 5.0 CaCl2, 0.03 $CaNa_2EDTA$, 11.5 glucose. (Webb and Bohr, 1978; Ebeigbe and Aloamaka, 1987).

Endothelium removal was effected by gently rubbing the internal lining of the arterial rings with a rough pair of forceps. Viability of the endothelium was established by a 60-70% relaxation response to 10^{-5}M Acetylcholine in 10^{-7}M phenylephrine-contracted endothelium-intact rings. Rings were given an initial load of 2g and the PSS was bubbled with 95% O_2 and 5% CO_2 gas mixture at 37^0C and pH of 7.4. An equilibration time of 90 minutes was allowed.

The membrane-stabilizing effect of calcium was examined by increasing the PSS Ca^{2+} concentration from 5.0 to 25mM during exposure for 30 minutes to low-bicarbonate PSS in both endothelium-intact and endothelium-denuded rings pre-contracted using EC_{70} concentration of phenylephrine. The influence of 10^{-6}M Methylene blue (MB) on the relaxation response induced by 25mM Ca^{2+} was examined by addition of MB for 10 minutes to the end of the 30-minute low-bicarbonate PSS exposure.

Statistical analysis

Data are presented as Means ± SEM. Student's t test was used in analysing results and graphical analyses were effected using Microcal Origin 5.0 software (Microcal Software Inc, Northampton, USA). Values of p less than 0.05 were considered significant and n values denote the number of animals from which vessels were obtained.

RESULTS

Relaxation response to Acetylcholine

The relaxation response induced by 10^{-5}M Acetylcholine following pre-contraction induced by EC_{70} concentration of phenylephrine was examined in aortic rings with intact or denuded endothelium, in order to assess the functional state of the endothelium. Ach relaxation of about 40% was observed only in rings with intact endothelium (Fig. 1).

Membrane stabilization

The relaxation response induced by increasing the PSS Ca^{2+} concentration to 25mM (due to membrane stabilization) is shown in Fig. 2. The magnitude of the relaxation response to Ca^{2+} was 38 ± 2.3% (Fig 2a). Exposure of the rings for 10 minutes to 10^{-6}M methylene blue resulted in a significant attenuation of the relaxation response (Figs 2b, 2c). Relaxation responses to 25mM Ca^{2+} were observed in both endothelium-intact as well as endothelium-denuded rings; however, there was a significant attenuation in endothelium-denuded rings (Fig. 2).

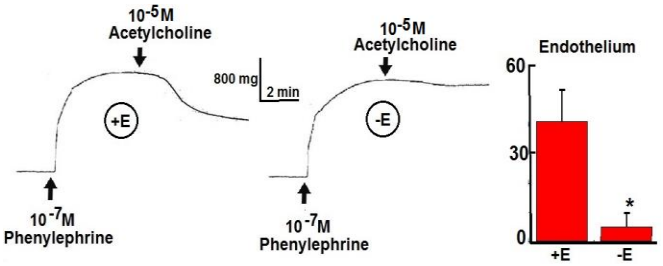

Fig. 1: Representative tracings showing the relaxation response to 10^{-5}M Acetylcholine in a phenylephrine pre-contracted ring with intact endothelium ((+E) and the attenuated relaxation response in endothelium-denuded ring (-E). Mean values are summarised in the histogram; asterisk denotes significant difference; $p<0.05$.

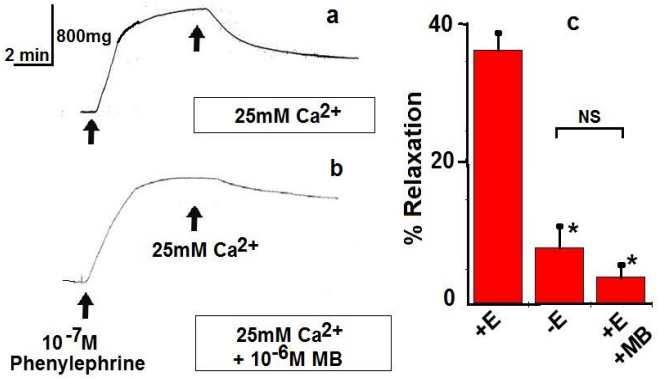

Fig. 2. Relaxation response induced by 25mM Ca^{2+} in 10^{-7}M phenylephrine pre-contracted aortic rings in control (a) and following 10-minute exposure to 10^{-6}M methylene blue (MB, b). Histogram (c) shows summary of data highlighting the attenuated Ca^{2+} relaxation responses in endothelium-denuded and in MB-treated rings. Values are Means ±SEM; n = 10.

DISCUSSION

Various studies have reported the beneficial effects of dietary Ca^{2+} supplementation in the enhancement of blood flow and that restricted Ca^{2+} intake results in decreased plasma Ca^{2+} level, uterine blood flow, increased BP and elevated urinary protein (Adamova et

al, 2009). The mechanism of the reduced blood flow and attenuated vascular reactivity due to excess Ca^{2+} has been suggested to involve the endothelial cell-dependent NO-guanylate cyclase pathway (Ezimokhai and Osman, 1998).

Report by Webb (1982) showed that D-600 antagonized the inhibitory effect of elevated calcium on contractile responses of the rat tail artery by blocking the transmembrane movement of calcium as well as interfering with membrane sites which mediate the stabilizing effect of Ca^{2+}. Observation in the present study, that relaxation induced by elevated Ca^{2+} is attenuated in rabbit aortic rings with denuded endothelium suggests a role for vascular endothelium in the membrane-stabilizing action of Ca^{2+} ions and is in line with observation in rat aortic smooth muscle (Wu and Bohr, 1991).

Studies have shown that agents that alter EDRF action or formation are similar in action to those that alter the effect of cyclic GMP. Also, the formation/release of EDRF is Ca^{2+}-dependent and is blocked by methylene blue which inhibits soluble guanylate cyclase. MB is also known to inactivate nitric oxide (NO) by generation of superoxide anions (Marczin et al., 1992).

EDRF is known to stimulate soluble guanylate cyclase which elevates the level of guanosine 3':5'-cyclic monophosphate (cyclic GMP) in vascular smooth muscle (Griffith et al., 1985) and induces vasorelaxation by activation of Ca^{2+}activated ATPases (Ignarro and Kadowitz, 1985).

The attenuation of Ach- and Ca^{2+}-induced relaxation responses by endothelium removal and methylene blue gives further credence to the involvement of the endothelium in mediating the relaxation responses.

In conclusion, the results indicate that the relaxation response induced by high concentration of Ca^{2+} ions in rabbit aortic smooth muscle is endothelium-dependent and is possibly mediated by the NO-guanylyl cyclase pathway.

REFERENCES

Adamova Z., S. Ozkan, and R.A. Khalil (2009). Vascular and Cellular Calcium in Normal and Hypertensive Pregnancy. *Curr Clin Pharmacol.* 4(3): 172–190.

Bolton T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscles. *Physiol. Rev.* 59: 606-718.

Ebeigbe A.B. and Aloamaka C.P. (1987). Role of endothelium in magnesium-induced relaxation of rat aorta. *Res. Exp. Med.* **187:** 25-31.

Edwards D.H., Griffith T.M., Henderson A.H., Lewis M.J. and Newby A.C. (1985). Production of endothelium-derived relaxant factor is both ATP and calcium dependent. *Br. J. Pharmacol*.**84:**64.

Ezimokhai M and N. Osman (1998). Calcium supplementation is associated with endothelium dependent attenuation of vascular smooth muscle reactivity in normotensive pregnant and nonpregnant rats. *Am J Hypertens.* 11(1): 88-96.

Fleming I., Bauersachs J., Fisslthaler B. and Busse R. (1998). Ca^{2+} -independent activation of the endothelial nitric oxide synthase in response to tyrosine phosphatase inhibitors and fluid shear stress. *Circ Res*.**82:**686–695.

Giles T.D. (2006). Aspects of nitric oxide in health and disease: a focus on hypertension and cardiovascular disease. *J. Clin. Hypertens.* (Greenwich). 12 Suppl 4: 2-16.

Griffith T.M., Edwards D.H., Lewis M.J.and Henderson A.H. (1985). Evidence that cyclic guanosine monophosphate (c-GMP) mediates endothelium-dependent relaxation. *Eur. J. Pharmacol*.**112**: 195-202.

Loeb A.L., Izzo N.J., Johnson R.M., Garrison J.C. and Peach M.J. (1988). Endothelium-derived relaxing factor release associated with increased endothelial cell inositol triphosphate and intracellular calcium. *Am. J. Cardiol*. **62:** 36G-40G.

Long C.J. and Stone T.W. (1985). The release of endothelium-derived relaxant factor is calcium dependent. *Blood Vessels*,**22:**205-208.

Marczin N., U.S. Ryan and J.D. Catravas (1992). Methylene blue inhibits nitrovasodilator- and endothelium-derived relaxing factor-induced cyclic GMP accumulation in cultured pulmonary arterial smooth muscle cells via generation of superoxide anion. *J Pharmacol Exp Ther*. 263: 170–179.

Sanders D.B., T. Kelley, and D. Larson (2000). The role of nitric oxide synthase/nitric oxide in vascular smooth muscle control. *Perfusion* 15(2): 97-104.

Webb R.C. (1982**).** D-600 and the membrane stabilizing effect of calcium in vascular smooth muscle. *Pharmacology* 25(5): 250-61.

Webb R.C. (2003). Smooth muscle contraction and relaxation. *Adv. Physiol. Educ.* 27(1-4): 201-6.

Webb R. C. and Bohr D.F. (1978)**.** Mechanism of membrane stabilization by calcium in vascular smooth muscle. *Am. J. Physiol*. **235:** 227-232.

Wu C.C. and D.F. Bohr (1991). Mechanisms of calcium relaxation of vascular smooth muscle. *Am J Physiol.* 261:H1411-6.