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Research Article

Altered extracellular magnesium and variations in vascular smooth muscle responses to agonists

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ABSTRACT

Background: There is a paucity of information on the heterogeneity of vascular smooth muscles in their responses to agonists following exposure to varying extracellular magnesium, $[Mg^{2+}]_0$. The present study was designed to examine, comparatively, the influence of variations in $[Mg^{2+}]_0$ on vascular smooth muscles of rabbit aortic, carotid and femoral arteries. **Methods**: Contractile responses induced by Phenylephrine (PE), Histamine (HIST) and 5-HydroxylTryptamine (5-HT) were examined on 2mm ring segments of the arteries 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 2g. Cumulative concentration responses to the agonists were studied in normal PSS (control) and following 30 minutes exposure to Mg^{2+}-free or high-Mg^{2+} (4.8mM) PSS. Contractile responses were expressed as percentage of 80 mM K^+ contractions in normal PSS. **Results:** Maximal contractions (E_{max}) induced by PE, HIST and 5-HT compared with high K^+ contraction in the various preparations were differentially altered ($p<0.05$) following exposure to varying $[Mg^{2+}]_o$. **Conclusion:** Based on the sensitivity (EC_{30}) and potency (EC_{50}) values for the dose-response curves of the agonists, we report that vascular smooth muscles of rabbit aortic, carotid and femoral arteries demonstrate considerable variability in their responses to altered $[Mg^{2+}]_o$.

INTRODUCTION

Assessment of reactivity in vascular smooth muscle of blood vessels from different anatomical sites is particularly important in understanding the mechanisms of regional blood flow regulation and cardiovascular function in health and disease states. Considerable heterogeneity has been reported to exist in the responses of vascular smooth muscles to various vasoactive agents (Daemen and De Mey, 1995). Experimental, clinical and epidemiological studies have shown that changes in magnesium concentration affect blood flow, vascular reactivity, and blood pressure (Pascal et al., 1997; Ebeigbe and Aloamaka,1986; Saris et al., 2000; Touyz, 2008; Goytain and Quamme, 2008)). Low or high $[Mg^{2+}]_o$ have been reported to enhance or attenuate (respectively), contractile response to agonists in different vascular beds (Altura and Altura 1978a; Altura et al., 1987) however, there is a paucity of information on the comparative effects of altered $[Mg^{2+}]o$ responses to agonists in different vascular preparations. The goal of the present study was to examines the effects of variations in $[Mg^{2+}]o$ on contractile responses induced by phenylephrine (PE), histamine (HIST) and 5-hydroxytryptamine (5-HT) on vascular smooth muscle of rabbit aorta, carotid and femoral arteries.

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MATERIALS AND METHODS

Tissue preparation and Protocol:

Arterial segments of the aorta, carotid and femoral arteries were obtained from adult New Zealand rabbits which were sacrificed by stunning and bleeding and placed in physiological salt solution (PSS) of the following composition (mM): NaCl 119, KCl 4.7, $NaHCO_3$ 24.9, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.6, glucose 11.5. The arteries were cleaned of adhering

connective tissues and cut into 2-3mm rings. The rings were suspended between 2 L-shaped wire loops in 20 ml organ baths containing PSS. The upper loop was attached to a Grass Model FT03 force transducer connected to a Grass Model 7P polygraph (Grass Instruments Co., Quincy, MA, USA) while the lower loop was fixed to the base of the organ bath. An initial load of 2g was applied. The PSS was bubbled throughout with 95% O_2 - 5% CO_2 gas mixture with the pH and temperature maintained at 7.4 and 37ºC respectively. 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 phenylephrine or histamine were evaluated.

Concentration-response to agonists:

Cumulative concentration-response tests ($1x10^{-9}$ to 2.5 $x10^{-4}$) to each of the 3 agonists (PE, HIST and 5-HT) were examined in normal PSS (control) (n = 8); as well as following 30 minutes exposure to Mg^{2+}-free or high-Mg^{2+} (4.8 mM) PSS (n = 8).

Chemicals

The following drugs and chemical reagents were used: Phenylephrine hydrochloride (Sigma USA), histamine hydrochloride (Merck AG, Germany) and 5-Hydroxytryptamine creatinine sulphate (Sigma USA); and prepared fresh by dissolving in distilled water.

Statistical Analysis

Data are presented as Means ± SEM (standard error of means); n represents the number of rabbits from which arterial rings were obtained. EC_{50} (concentrations producing 50% maximal response) were determined graphically. Comparison of the means was effected using the Student's t-test, ANOVA and Microcal origin 8.0 statistical package. P - Values less than 0.05 ($P<0.05$) were considered statistically significant for two independent variables (test and control).

RESULTS

$(Mg^{2+})_o$ and dose-response to agonists

Exposure of arterial tissues to varying $(Mg^{2+})o$:

Exposure of arterial rings to Mg^{2+} -free or high-Mg^{2+} PSS resulted in enhanced or attenuated contractions induced by the 3 agonists (PE, HIST and 5-HT) as shown in Figs 1-3.

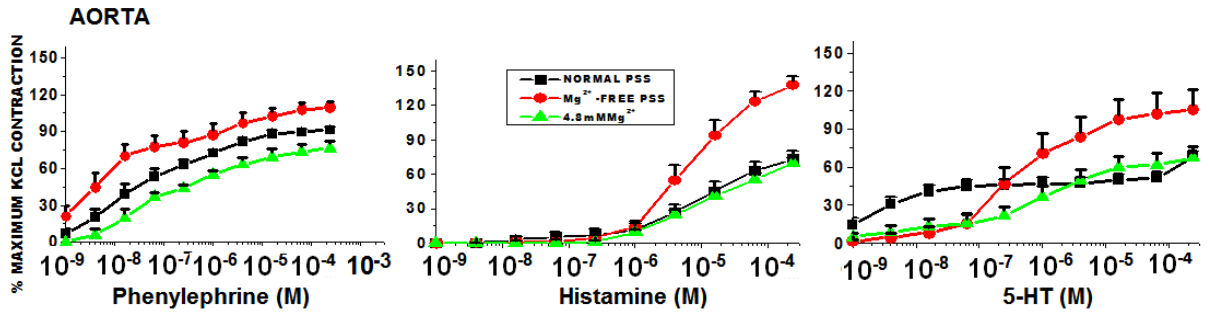

Fig.1. Dose response to agonists flowing exposure to varying $(Mg^{2+})_o$ PSS in aortic rings; n = 8. $(Mg^{2+})_o$ exposure shifted the curves left-ward and enhanced maximal contractions whereas high-Mg^{2+} (4.8mM) PSS resulted in a right-ward shift and attenuated maximal contractions. Values are means ± SEM

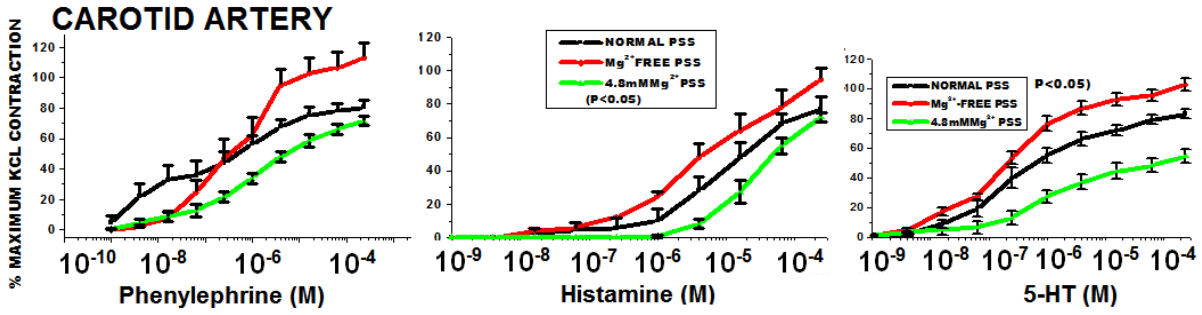

Fig.2. Dose response to agonists flowing exposure to varying $(Mg^{2+})_o$ PSS in carotid artery rings; n = 8. $(Mg^{2+})_o$ exposure shifted the curves left-ward and enhanced maximal contractions whereas high-Mg^{2+} (4.8mM) PSS resulted in a right-ward shift and attenuated maximal contractions. Values are means ± SEM

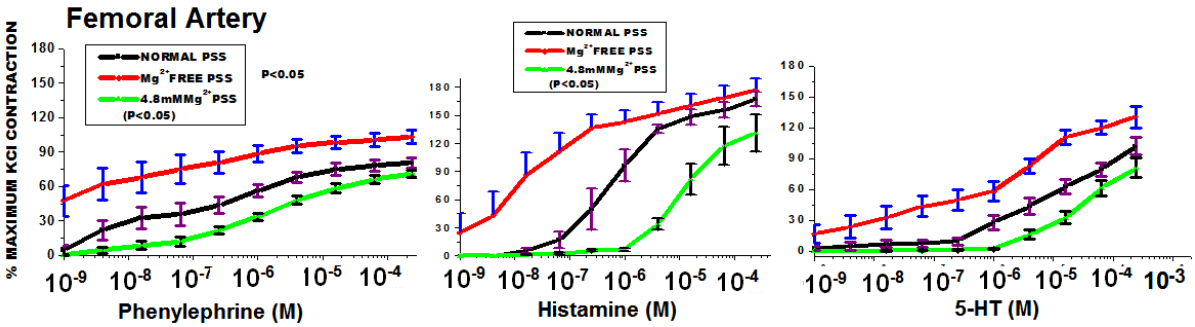

Fig.3. Dose response to agonists (PE, HIST, & 5-HT) flowing exposure to varying $(Mg^{2+})_o$ PSS in femoral artery rings; n = 8. $(Mg^{2+})_o$ exposure shifted the curves left-ward and enhanced maximal contractions whereas high-Mg^{2+} (4.8mM) PSS resulted in a right-ward shift and attenuated maximal contractions. Values are means ± SEM

In aortic rings, the magnitude of enhancement of contractions was in the order: HIST > 5-HT > PE whereas the order of attenuation by 4.8mM Mg^{2+} was: PE > 5-HT > HIST.

Figs 2 and 3 show the enhancement and attenuation (respectively), of contractile responses to agonists (PE, HIST, & 5-HT) in rabbit carotid artery (n = 8), and femoral artery (n=8) rings following exposure to Mg^{2+}-free or high Mg^{2+} PSS.

The EC_{50} values for contractile responses by the three agonists and the differential potentiation/attenuation by Mg^{2+}-free/high- Mg^{2+} exposure in aortic, carotid and femoral arteries are shown in Tables 1-3.

The EC_{50} (M) and E_{MAX} (%) values for agonist-induced contractile responses and the influence of varied $(Mg^{2+})_o$ show that the enhancement by $(Mg^{2+})_o$ was in the order - for PE: Femoral artery> aorta>carotid artery; for HIST: Femoral artery> carotid>aorta and for 5-HT: Aorta> femoral>carotid artery; whereas attenuation by high-Mg^{2+} PSS the order - for PE and HIST: Femoral artery>aorta>carotid artery; and for 5-HT: Carotid artery> aorta>femoral artery

Table 1. EC_{50} [M] & E_{MAX} (%) values for Phenylephrine contractions

Tissue	PE	EC_{50} [M]		E_{MAX} [%]		
	NORMAL (M)	Mg^{2+} FREE (M)	HIGH Mg^{2+} (M)	NORMAL (%)	Mg^{2+} FREE (%)	HIGH Mg^{2+} (%)
Aorta	$8.06 \pm 0.45 X10^{-8}$	$5.33 \pm 0.50x10^{-9}$	$1.04 \pm 0.24 \times 10^{-6}$	9.14 ± 2.47	109.67 ± 4.69	76.45 ± 5.39
Carotid	$3.20 \pm 0.02 X10^{-6}$	$2.02 \pm 0.03 \times 10^{-7}$	$1.79 \pm 0.06 x10^{-5}$	80.42 ± 4.65	108.48 ± 5.68	71.31 ± 3.00
Femoral	$4.00 \pm 0.03 X10^{-8}$	$5.57 \pm 0.03 \times 10^{-9}$	$1.43 \pm 0.03 \times 10^{-7}$	138.79 ± 10.07	151.79 ± 15.66	126.06 ± 16.48

Values are means **± SEM;** n =8.

Table 2. EC_{50} [M] & E_{MAX} (%) values for Histamine contractions

Tissue	HIST	EC_{50} [M]		E_{MAX} [%]		
	NORMAL (M)	Mg^{2+} FREE (M)	HIGH Mg^{2+} (M)	NORMAL (%)	Mg^{2+} FREE (%)	HIGH Mg^{2+}(%)
Aorta	$2.20 \pm 0.10 \times 10^{-5}$	$9.80 \pm 0.03 \times 10^{-6}$	$6.00 \pm 0.05 \times 10^{-5}$	73.25 ±7.27	137.73 ± 7.79	69.50 ± 4.68
Carotid	$1.40 \pm 0.02 \times 10^{-5}$	$8.00 \pm 0.01 \times 10^{-6}$	$8.50 \pm 0.06 \times 10^{-5}$	76. 68 ± 7.59	94.70 ± 6.79	74.17 ± 3.85
Femoral	$4.20 \pm 0.01 \times 10^{-7}$	$5.00 \pm 0.02 \times 10^{-8}$	$1.24 \pm 0.02 \times 10^{-5}$	159.33 ±15.86	176.74 ± 12.28	130.94 ± 19.35

Values are means **± SEM;** n =8.

Table 3. EC_{50} [M] & E_{MAX} (%) values for 5-HTcontractions

Tissue	5-HT	EC_{50} [M]		E_{MAX} [%]		
	NORMAL (M)	Mg2+-FREE(M)	HIGH Mg^{2+} ((%)	NORMAL (%)	Mg^{2+} FREE (%)	HIGH Mg^{2+} (%)
Aorta	$2.4 \pm 0.07 \times 10^{-6}$	$6.07 \pm 0.03 \times 10^{-7}$	$2.20 \pm 0.09 \times 10^{-5}$	67.82 ± 4.74	105.39 ± 16.16	67.47 ± 8.56
Carotid	$7.30 \pm 0.03 \times 10^{-7}$	$2.57 \pm 0.02 x10^{-9}$	$1.70 \pm 0.04 x10^{-6}$	83.14 ± 3.35	102.73 ± 4.21	54.79 ± 4.64
Femoral	$1.84 \pm 0.06 \times 10^{-5}$	$4.40 \pm 0.05 \times 10^{-6}$	$1.66 \pm 0.05 \times 10^{-4}$	101.95 ± 8.17	154.00 ± 8.30	80.81 ± 9.33

Values are means **± SEM;** n =8.

DISCUSSION

The results of the present study are in line with previous reports (Altura and Altura, 1990; Eun et al., 2004; Goytain and Quamme,2008) that withdrawal of $(Mg^{2+})_o$ enhances, while elevation in $(Mg^{2+})_o$ depresses contractile responses of vascular smooth muscles. We have examined contractile responses induced by three agonists and their modulation by altered $(Mg^{2+})_o$ vascular reactivity to vasoconstrictor agents: phenylephrine, histamine and 5-HT, as well as modification of their responses following exposure to varying $(Mg^{2+})_o$, in a variety of blood vessels. Based on

the EC_{50} values for the 3 agonists studied, it is clear that a considerable heterogeneity exists in the contractile responses induced by the agonists following exposure to varied $(Mg^{2+})_o$ media. A number of studies have shown that regional and species variations exist in the responses of isolated arteries to a variety of vasoactive agents (Toda and Miyazaki, 1978; Toda et al, 1990; Daemen and De Mey, 1995). Most of the previously reported studies have focused on blood vessels obtained from the rabbit, dog, or monkey and have not examined, comparatively, the modulation by $(Mg^{2+})_o$ of contractile responses to agonists in vascular smooth muscle from different regional sites in the same animal species.

The agonists examined in this study elicit vascular smooth muscle contraction by mobilizing intracellular calcium pools as well as stimulating Ca^{2+} influx through receptor-operated channels (Bolton, 1979; Ebeigbe, 1982). Therefore, the observations and findings from our data could be interpreted to indicate that Mg^{2+} might interfere with the binding of these agonists to their various receptors in rabbit arterial blood vessels as has been reported by other workers (Altura and Turlapaty, 1982). A variety of mechanisms may account for the differential modulation by $(Mg^{2+})_o$ of agonist-induced contractions of arterial preparations: previous studies indicate that magnesium ion can either increase or decrease the affinity of various agonists for their specific receptors in different types of vascular smooth muscle (Somlyo et al., 1966; Altura and Altura, 1971., Goldstein and Zsoter, 1978). Furthermore, differences in responsiveness of these agonists and modulation by Mg^{2+} of contractile responses in arteries from different regional sites may result from differences in the properties of regulation of the contractile processes (Eun et al., 2004; D'Angelo et al., 1992), properties of vascular agonists specific receptors and /or diversity of smooth muscle types; as well as up or down regulation of vascular smooth muscle population density in the length and regional vascular beds (Miwa and Toda,1994, Bascands et al., 2001). Also, $(Mg^{2+})_o$ impairs Ca^{2+} influx into vascular smooth muscle cells and also acts intracellularly as Ca^{2+} antagonist (Turlapaty et al 1981; Altura and Altura, 1983), thereby modulating the vasoconstrictor action of $(Ca^{2+})_i$, a major determinant of vascular smooth muscle contraction (Zheng et al, 2011).

CONCLUSION

In conclusion, vascular smooth muscle of rabbit aorta, carotid and femoral arteries exhibit considerable heterogeneity in their contractile responses to different agonists and modulation by extracellular magnesium concentration. Although the precise mechanisms for the differential effects reported are not clear, an understanding of vascular reactivity pattern across regional beds is of vital importance for regional blood flow regulation and cardiovascular function.

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