

VITAMIN C ENHANCES RELAXATION OF RAT AORTIC RINGS INDUCED BY K^+ AND Mg^{2+}

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Summary This study was designed to determine the effects of administration of vitamin C on the relaxation of vascular smooth muscle to K^+ and Mg^{2+} . The experiments were performed in two groups of Sprague-Dawley rats aged 6 weeks. Both control and test groups received rat chow and normal drinking water. In addition, the test group received vitamin C (100 mg/kg/day) by intragastric administration for 8 weeks. At the end of this period aortic rings were obtained from both groups and used for isometric recordings. The relaxation responses to K^+ and Mg^{2+} after precontraction with noradrenaline (10^{-7} M) were higher in the vitamin C-treated group compared with the control group. There was no difference in the relaxation responses to Mg^{2+} following K^+ (40 mM) precontraction in the two groups. The enhanced relaxation to K^+ in vitamin C rats suggests an increase in vascular Na^+-K^+ ATPase activity. The increase in relaxation to Mg^{2+} following NA precontraction, which was not observed with K^+ precontraction, suggests that vitamin C enhances the mechanism of Mg^{2+} -induced relaxation following receptor-mediated, but not depolarization-dependent contraction.

KEY WORDS: Vitamin C, Potassium, Magnesium, Vascular reactivity

Introduction

Intracellular Ca^{2+} concentration in vascular smooth muscle is dependent on the activity of the Na^+-K^+ pump (Bohr et al., 1991). Studies have shown depressed Na^+-K^+ ATPase activity in hypertension (M'Buyamba et al., 1994). Although the exact mechanism responsible for the depressed Na^+-K^+ ATPase activity remains unknown, oxidative stress has been suggested as a contributory factor (Huang et al., 1992). Free oxygen radicals are known to cause damage to Na^+-K^+ ATPase (Boldyrev et al., 1996), and antioxidants may enhance Na^+-K^+ ATPase activity by inactivating these reactive oxygen species in vascular smooth muscle.

Magnesium is an important modulator of vascular function. It causes vascular relaxation (Yang et al., 2000), and is used in the management of preeclampsia (Sibai, 1990). Animals with magnesium deficiency acquire hypertension (Bertholet & Wester, 1983), and chronic administration of magnesium delays the onset of hypertension in spontaneously hypertensive rats (Makynen et al., 1995). Reports suggest that the mechanism of vascular relaxation by Mg^{2+} involves the nitric oxide, prostanoids and inhibition of Ca^{2+} entry in vascular smooth muscle cells (Ebeigbe & Aloamaka, 1987; Longo et al., 2001). Free radicals decrease nitric oxide and prostacyclin bioavailability in the endothelium and thereby impair vasodilation (Tomaino & Decker 1998).

The present study therefore examined the effects of chronic vitamin C administration on the relaxation responses of rat aortic rings in response to potassium and magnesium ions.

Materials And Methods

Male Sprague-Dawley rats weighing 120-150 g and aged 6-8 weeks were used for this study. They were randomly divided into two groups: experimental group, which received oral administrations of vitamin C (100 mg/kg/day; Sigma Chemical Co, UK) for 8 weeks, and control group, which received distilled water as vehicle. All animals had free access to food and water.

Preparation of Aortic Rings

At 8 weeks, the rats were killed by cervical dislocation and the thoracic aorta immediately removed, freed of adherent tissue and placed in normal physiological salt solution (PSS) of the following composition (mM) NaCl 119, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 14.9, $CaCl_2$ 1.6, glucose 11.5. The solution was continuously bubbled with 95% O_2 -5% CO_2 gas mixture at 37°C. The aorta was cut into 2 mm rings and suspended between 2 stainless steel L-shaped rods in a 20 ml tissue bath containing PSS. One rod was hooked to the base of the bath and the other was connected to a force displacement transducer (Grass FT 03) which was in turn connected to a Grass model 7D polygraph for recording of isometric contractions. The rings were allowed to equilibrate for 90 min under a resting tension of 2 g

during which it was stimulated three times with NA (10^{-7} M) at 30 min intervals.

Relaxation Response to Potassium Chloride

The aortic rings were incubated in the K^+ -free PSS for 15 min before precontraction with NA (10^{-7} M). When the contraction had peaked, KCl (0.05 – 10 mM) was added cumulatively.

Relaxation Response to Magnesium Sulphate

The relaxation response to $MgSO_4$ was carried out by incubating the aortic rings in Mg^{2+} -free PSS for 30 min before precontraction with NA (10^{-7} M). The contraction was allowed to plateau before cumulative addition of $MgSO_4$ (0.05 – 10 mM).

Statistics Data are presented as means \pm SEM. The agonist concentration which produced 50% of maximal relaxation (EC_{50}) was calculated for each ring. Comparison of data between control and experimental groups was done with Student's unpaired t test. P values less than 0.05 were considered significant.

Results

Relaxation Response to Potassium Chloride

Figure 1 shows the relaxation response curves of aortic rings to KCl in K^+ -free PSS. Rings from vitamin C treated rats showed enhanced relaxation to KCl when compared with those of control rats. There was a significant difference in the sensitivity of the rings from the vitamin C rats as shown by the lower EC_{50} value in this group compared with the control group (Table 1).

Table 1: EC_{50} (mM) values for KCl and $MgSO_4$ from aortic rings of control and vitamin C-treated rats

	Control	Vitamin C
KCl	1.93 ± 0.45	$0.21 \pm 0.14^*$
$MgSO_4$ (NA)	5.67 ± 0.55	$3.96 \pm 0.31^*$
$MgSO_4$ (KCl)	6.62 ± 0.23	5.91 ± 0.39

Values are mean \pm SEM. n = 6 per group. *P<0.05 compared with control.

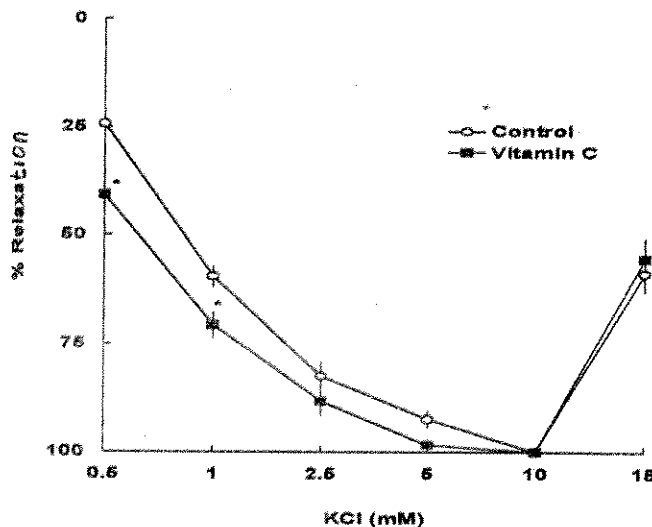


Fig. 1. Concentration-response curves to KCl following precontraction with noradrenaline (10^{-7} M) in control and vitamin C-treated rats. Mean \pm SEM of 6 experiments. *P<0.05

Relaxation Response to Magnesium Sulphate

The relaxation response to MgSO_4 in rings from rats of the two groups were significantly different following precontraction with NA ($P < 0.05$) (Fig. 2), but were not different following precontraction with KCl (Fig. 3). In rings precontracted using NA, the EC_{50} value for the relaxation response to MgSO_4 from the vitamin C-treated rats was significantly ($P < 0.05$) less than that of the control rats (Table 1). In rings precontracted with KCl, the EC_{50} values for both groups were not significantly different.

Discussion

The present study shows that aortic ring preparations from rats pretreated with vitamin C show enhanced relaxation to low concentrations of KCl. The ability of precontracted blood vessels to relax to slight increases in concentration of KCl is well documented (Adegunloye & Sofola, 1998). It used as a functional measure of the $\text{Na}^+\text{-K}^+$ ATPase activity (Webb & Bohr, 1978). An increase in $\text{Na}^+\text{-K}^+$ ATPase activity leads to a decrease in intracellular Ca^{2+} concentration and ultimately a reduction in vascular tone. Biochemical studies have shown that antioxidants may improve $\text{Na}^+\text{-K}^+$ ATPase activity in different tissues (Yesilkaya & Yegin, 1998). The greater KCl relaxation in aortic rings from vitamin C-treated rats is suggestive of altered $\text{Na}^+\text{-K}^+$ ATPase activity.

Numerous studies show that elevated or lowered extracellular magnesium concentration

results in decreased or increased contractile responses respectively in vascular smooth muscle (Ebeigbe & Aloamaka, 1987; Longo et al., 2001). However, the mechanisms involved in the relaxation of blood vessels to Mg^{2+} are yet to be fully elucidated. Magnesium is considered a calcium channel blocker and may cause relaxation by inhibiting the entry of Ca^{2+} from the extracellular fluid. It may also inhibit the release of Ca^{2+} from intracellular stores (Sjogren & Edvinsson, 1988). There are also reports that show the involvement of the endothelium in the relaxation response to Mg^{2+} . Ebeigbe & Aloamaka (1987) observed that the influence of the endothelium was dependent on the agent used to induce tone. They found that relaxation was enhanced in the presence of the endothelium only in vessels precontracted with NA, but not in vessels precontracted with KCl. The enhanced vascular relaxation in the vitamin C-treated rats suggests that the antioxidant vitamin may enhance mechanisms involved in the relaxation response to Mg^{2+} following receptor-mediated contraction using NA, but not following depolarization-dependent contraction using KCl. This may involve protection of endothelium-derived factors which are inactivated by free radicals.

In conclusion, it is suggested that long-term vitamin C administration may increase $\text{Na}^+\text{-K}^+$ ATPase activity in vascular smooth muscle, and enhance the mechanism of vascular relaxation induced by Mg^{2+} following receptor-mediated, but not depolarization-dependent contraction.

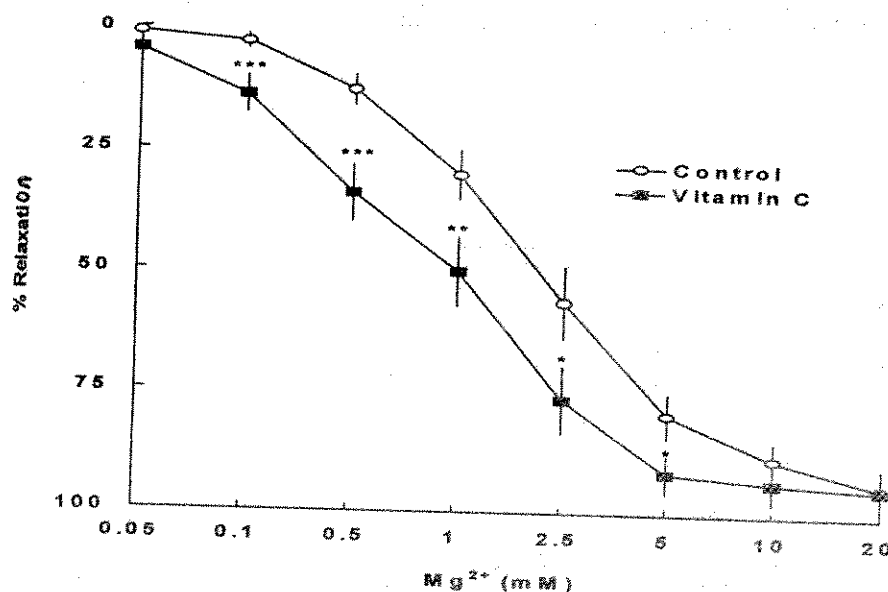


Fig. 2: Concentration-response curves to MgSO_4 following precontraction with noradrenaline (10^{-7} M) in control and vitamin C-treated rats. Mean \pm SEM of 6 experiments. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. control.

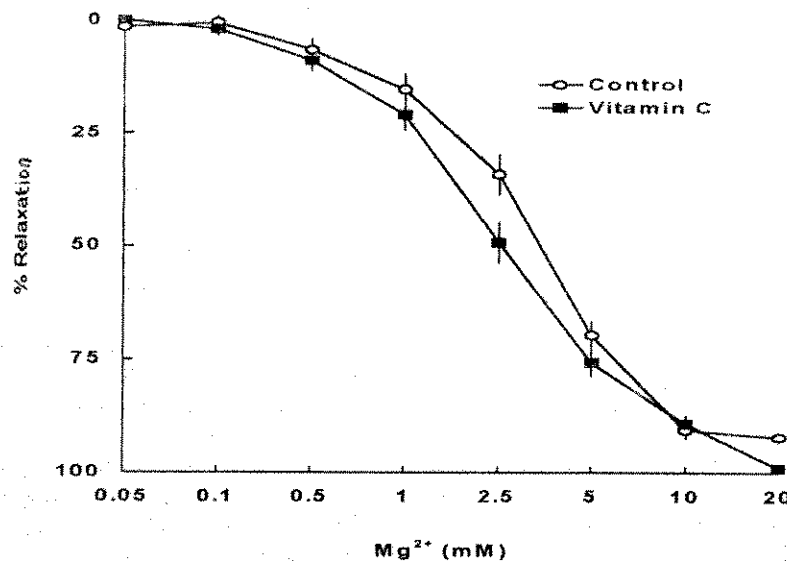


Fig 3: Concentration-response curves to MgSO_4 following precontraction with KCl (40 mM) in control and vitamin C-treated rats. Mean \pm SEM of 6 experiments.

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Received: April 14, 2002

Accepted: November 13, 2002