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Depressed magnesium-induced relaxation in aortic rings from alloxan-induced diabetic rats

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ABSTRACT The relaxation of aortic smooth muscle to variations in extracelluar magnesium concentration (Mg^{2+}) was studied in normal and diabetic rats. The relaxation response induced by raised extracellular Potassium concentration was attenuated in aortic rings from diabetic rats. The results suggest that Mg^{2+} - induced depression of rat aortic smooth muscle contraction could be associated with release of endothelium-derived release factor.

Key Words: Alloxan-induced diabetes; Aortic muscle; Aortic rings; Magnesium; Ralaxation.

Introduction

Magnesium (Mg^{2+}) ion is a divalent cation normally existing in the body fluids. The relationship of Mg^{2+} with the contractile responses of vascular smooth muscle has aroused much interest.

Lower or increased extracullar magnesium concentration (Mg^{2+}) or results (respectively) in potentiation or attenuation of contractile responses in various vascular smooth muscle preparations (Altura and Altura, 1974; 1976; Krishnamurity and Mukherjee, 1981; Ebeiegbe and Aloamaka, 1986).

Acute hypomagnesemia in animals and humans is often associated with rises in blood pressure and elevations in peripheral vascular resistance in a variety of regions in the circulation (Altura and Altura, 1978; 1981). Hypermagnesemia, on the other hand, which is frequently noted in renal vascular disorders and circulatory shock, is often associated with hypotensive episodes (Altura and Altura, 1978). It has been demonstrated repeatedly that acute elevations in serum Mg^{2+} concentrations can produce profound and rapid vasolidatation and hypotension (Aikawa, 1971; Hazard and Wurmser, 1932).

The results of these previous works gave support to the ideal that reductions or elevations in serum levels of Mg^{2+} could be narrow or dilate, respectively arterial and arteriolar lumen sizes by enhancing or decreasing activity of circulating constrictors.

Altura *et al* (1982) showed that peripheral vessels which do not normally develop a spontaneous tone or basal tension such as intrapulmonary arteries and veins, or splanchnic arteries (i.e. hepatic, celiac, splenic, gastric, superior mesenteric) will not undergo vasospasm when $(Mg^{2+})o$ is lowered.

However, these same workers equally showed that vessels which normally develop spontaneous mechanical activity (e.g. cerebral, and coronary arteries, rat aortae and mesenteric artericles) would undergo

spasm of enhanced spike frequency and amplitude as the extracellular magnesium in their environment is reduced.

McNair *et al.* (1978); Wącker (1980); Altura and Altura, (1982); reported that these effects of magnesium on vascular smooth muscle cells could aid in explaining the association between certain vascular disease states and magnesium levels in the blood and tissues.

Several investigations point to a casual relation between decreased magnesium ion content of cardiac muscle and coronary ischaemic heart failure (Altura *et al*; 1982; Crawford and Crawford, 1967; Seeling, 1980).

Elevated extracellular magnesium concentration is believed to depress vascular contractility by inhibiting calcium influx as well as decreasing total exchangeable and membrane-bound calcium (Turlapaty *et al*; 1981). Magnesium also acts by displacing a functional pool of calcium on the smooth muscle cell membrane (Altura, 1978). In a related study, Ebeigbe and Aloamaka (1987) showed that magnesium induced relaxation of rat aortic smooth muscle is associated with the release of an endothelium-derived relaxing factor (EDRF).

A number of disease states (e.g. diabetes mellitus, essential hypertension, alcoholism, atherosclerosis) that result in elevation of blood pressure are often associated decreased serum levels of magnesium (Altura *et al*; 1979).

In progressive diabetes mellitus, not only is there an increased loss of urinary $_2$, but in addition there have been numerous reports of a progressive plasma hypomagnesemia (Aikawa, 1971).

The dilating actions of Mg^{2+} on diabetes vessels have received very little attention. Sjogren and Edinsson (1988) observed no differences in responses to Mg^{2+} on diabetes vessels. This present study examines the influence of magnesium on contractile responses of Alloxan-induced diabetic rat aortae.

Methods

Male Wistar rats initially weighing 120 - 140g aged 10 - 12 weeks were used for this study. They were randomly into controland diabetic groups. Each test animal was made diabetic by intraperitoneal of alloxan (40 mg/kg body weight) in citrate buffer.

All animals had free access to food and water and were monitored daily for the development of glycosuria by testing for the presence of reducing sugar in the urine. Diabetes was confirmed when blood glucose (obtained by cutting the tip of the tail) was 4 times in excess of the normal.

2 weeks after introduction of diabetes, rats were killed by stunning and their aortae quickly isolated, freed of adhering connective tissue with microdissecting forceps before it was removed and placed in a petri dish containing normal PSS. Blood was gently flushed out of the lumen of the aorta with a 1ml syringe attached to a 23 guage needle and containing normal PSS.

The aorta was cut into approximately 2mm ring segment and suspended between L-shaped fine stainless steel rod and a stainless steel hook. This was transferred into a 20ml jacketed organ bath containing normal physiological salt solution (NPSS) of the following composition (mm/L): NaCl, 1190; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2;$CaCl_2$, 1.6; $NaHCO_3$, 1.25mg; Glucose, 2.0gm and pH 7.4. the solution was continuously oxygenated with 95% O_2 and 5% CO_2 gas mixture and maintained at 37^{0}C. The hook was enchored to the base of organ bath and stainless steel rod was connected to an isometric force displacement transducer (FT.03) which was coupled to grass model 79D polygraph for recording tension. The tissue was allowed to equilibrate for 90 minutes under resting tension of 1.5g prior to the commencement of experiments. At the end of equilibration period the rings were exposed to Mg^{2+}-free 1.6mmol - 1^{-1} Ca^{2+} PSS for 30 minutes before being stimulated with 10^{-7} mol. 1^{-1}- NA. When the contraction was stable, Mg^{2+} was added to the bath cumulatively, resulting in the concentration - dependent relaxation. The magnitude of relaxation was expressed as a percentage of the initial contractile response to NA in Mg^{2+}-free PSS. Mg^{2+}- free PSS was prepared by excluding Mg^{2+} from the normal PSS.

Values are presented as means $\pm$ SEM. Comparison between control and diabetic group was made the student's unpaired t-test. P-values less than 0.05 were considered statistically significant. N denotes number of animals in each group.

Results

Diabetic rats had elevated blood glucose level when compared with agematched controls. Additionally, they exhibited other symptoms commonly associated with diabetes mellitus (e.g. polyuria, polydipsia and diarrhea).

Figure 1 is a representative tracing of the protocol used to study Mg^{2+} - induced relaxation. In all experiments, Mg^{2+} caused concentration - dependent relaxation following pre-contraction by Noradrenaline (NA).

Mg^{2+} - induced relaxation was significantly attenuated in rings from diabetic rats (Fig.2).

Discussion

Results of the present study show that the relaxation responses induced by raised extracellular magnesium concentration is significantly attenuated in aortic rings from diabetic rats.

Magnesium ion is known to interfere with Ca^{2+} binding and translocation and hence cause relaxation of vascular smooth muscle (Altura and Altura, 1974). In diabetes, contractile responses depend largely on influx of extracellular Ca^{2+} ions. Raised (Mg^{2+}) has been shown to depress vascular smooth muscle concentration by competition with Ca^{2+} at membrane sites (Ebeigbe *et al*; 1984). Earlier study reports that Mg^{2+} -induced depression of rat aortic smooth muscle contractions is associated at least, in part, with the release of an endothelium- dependent relaxant factor (EDRF) in receptor-mediated contractions (Ebeigbe & Aloamaka, 1987).

Abnormal vascular reactivity to various vasoactive agents has been reported in chemically induced diabetic animals. However, little is known about the molecular mechanism underlying the alterations in vascular smooth muscle function in diabetic animals.

The vascular endothelium modulates the contractility of isolated arterial segments by producing and releasing an endothelium-derived relaxant factor (EDRF) at rest or on stimulation by a number of vasodilators (Furchgott, 1983).

Endothelial dysfunction has been implicated in the pathogenesis of diabetic vascular disease: endothelium-dependent relaxations of isolated aortae from diabetic rabbits, in response to acetycholine and adenosine diphophate (ADP) are depressed; in contrast, relaxation responses to sodium nitoprusside (which acts directly on the smooth muscle cells) are unchanged (Testamariam *et al*; 1993). Other reports abound of impaired endothelial function in diabetes: attenuated acetycholine -induced relaxation in mesenteric resistance arteries of streptozotocin -induced diabetic rats (Taylor *et al*; 1992).

In conclusion, the present study reports that Mg^{2+} -induced depression of diabetic aortic smooth muscle contraction could be associated with impaired vascular endothelial function.

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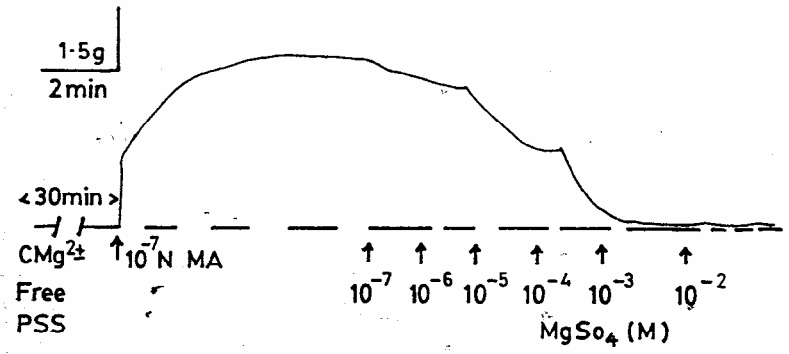

Fig. 1: A representative tracing illustrating Mg^{2+}-induced relaxation of a rat aortic strip pre-contracted by noradrenaline (NA).

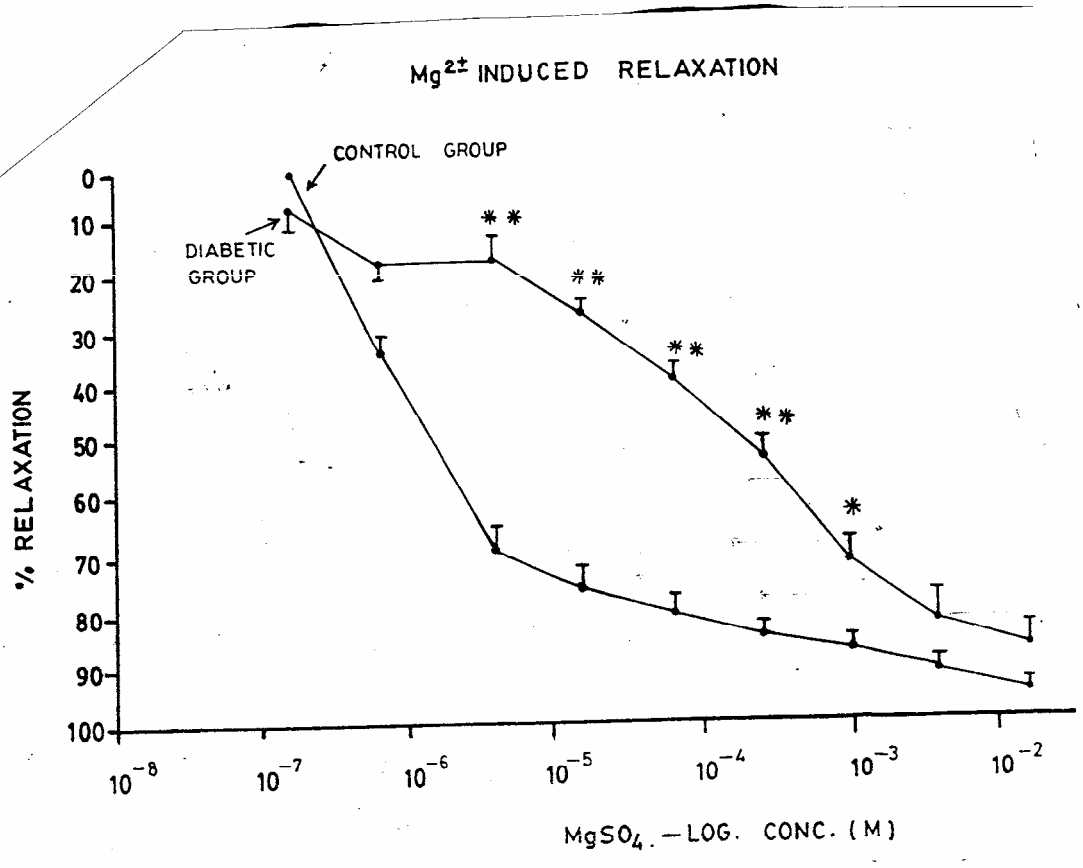

Fig. 2: Changes in Mg^{2+}-induced relaxation in aortic rings from alloxan-induced diabetic rats.

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