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Endothelium-dependent relaxation in coronary arteries requires magnesium ions

[1]Bella T. Altura & Burton M. Altura

Department of Physiology, State University of New York, Health Science Center at Brooklyn, New York 11203, U.S.A.

A great deal of interest has recently focused upon the mechanism(s) associated with the generation and action of endothelium-derived relaxant factors (EDRFs) in blood vessels. Since we have shown that extracellular magnesium ions ($[Mg^{2+}]_o$) are important in control of coronary vascular tone and reactivity, we wondered whether these divalent cations play any role in the generation or action of EDRF in coronary arterial smooth muscle. Using isolated canine coronary arterial rings, we have now found that removal of $[Mg^{2+}]_o$ inhibits the ability of these vascular preparations to relax when challenged with acetylcholine; in the absence of $[Mg^{2+}]_o$, the relaxation concentration-response curves for acetylcholine are shifted markedly to higher concentrations with small maxima. It, thus, appears that $[Mg^{2+}]_o$ is an important co-factor for acetylcholine-induced endothelium-dependent relaxation in canine coronary arteries. These findings support our previous hypothesis that dietary deficiency of Mg may be an important factor in aetiology of coronary vasopasm.

Introduction Considerable emphasis has recently been devoted to unravelling the mechanism(s) associated with generation and action of endothelium-derived relaxant factor (EDRF) discovered by Furchgott & Zawadzki in 1980 (Chand & Altura, 1981; Furchgott, 1983; Rapoport & Murad, 1983; Busse *et al.*, 1984; Griffith *et al.*, 1984; Cocks *et al.*, 1985). Denudation or destruction of the endothelial cells (EC) lining blood vessels leads to a loss of responsiveness to a wide variety of dilator substances including acetylcholine, bradykinin, arachidonic acid and ATP, among others. Also, the ability of many blood vessels to contract in response to a number of vasoconstrictors is enhanced after removal of EC. Although acetylcholine-induced endothelium-dependent relaxation in certain blood vessels is Ca^{2+}-dependent (Furchgott, 1983; DeFeudis, 1985), it is not clear whether Mg^{2+} is also important.

We (Altura & Altura, 1974; Turlapaty & Altura, 1980) and others (see Altura & Altura, 1985a, 1985b, for recent reviews) have found that reduction in the level of $[Mg^{2+}]_o$ can result in enhancement of coronary vascular tone, potentiation of coronary vasoconstrictors, as well as microcirculatory ischaemia and production of hypertension (Altura *et al.*, 1984). We now show that removal of $[Mg^{2+}]_o$ inhibits the ability of canine coronary arteries to relax in response to acetylcholine.

[1]Author for correspondence.

Methods Male mongrel dogs, weighing 12–20 kg, were anaesthetized with pentobarbitone sodium, 35 mg kg^{-1}. After thoractomy, the hearts were excised quickly and branches of left coronary arteries (o.d. of 0.3–0.5 mm) and circumflex arteries (o.d., 1–2 mm) were isolated and cut into 4–5 mm rings. These were suspended under 1.5 and 2.0 g tension, respectively, and incubated in 20 ml muscle chambers containing normal Krebs-Ringer bicarbonate solution (composition mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 10 and $NaHCO_3$ 25) at 37°C through which a mixture of O_2 (95%) and CO_2 (5%) was bubbled (Altura & Altura, 1974). Force of contraction was measured with Grass FT-03c force-displacement transducers and recorded on a Grass Model 7 polygraph. After 2 h incubation of the preparations under tension, the contractile effect of 80 mM KCl was determined in order to ascertain the maximal response. Subsequently, tissues were exposed to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$ UpJohn Co, Kalamazoo, 4.8×10^{-6} M) in order to produce sustained, submaximal contractions. Acetylcholine chloride (Sigma Co., St Louis) was then added in a cumulative dose manner to establish control relaxant responses. After washing and relaxation, the tissues were incubated in a Mg^{2+}-free Krebs-Ringer bicarbonate for 30–45 min, restimulated with $PGF_{2\alpha}$ and challenged with acetylcholine. A third concentration-response curve to acetylcholine was obtained upon re-introducing normal Krebs-Ringer bicarbonate containing Mg^{2+}. Where appropriate, mean (± s.e.mean) threshold, EC_{50} and maximal relations (%) were calculated.

Results Addition of acetylcholine to the physiological salt solution (containing Mg^{2+}), bathing the precontracted blood vessels, resulted in rapid and concentration-dependent relaxation in the coronary arterial rings (Figure 1a). Withdrawal of $[Mg^{2+}]_o$ from the bathing medium (Figure 1b) resulted in a profound

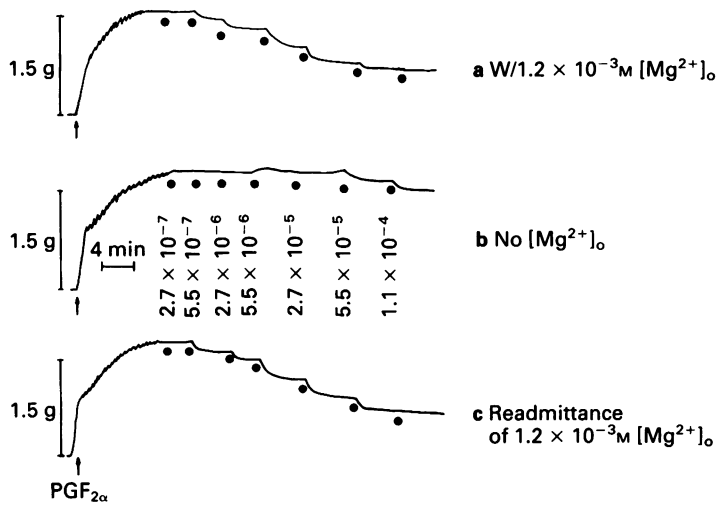

Figure 1 Acetylcholine induces concentration-dependent relaxation of canine coronary artery, provided the bathing media contains $[Mg^{2+}]_o$ (panels (a) and (c)). Removal of the $[Mg^{2+}]_o$ results in loss of generation of EDRF in response to acetylcholine (b). Readmittance of $[Mg^{2+}]_o$ restores relaxation response to acetylcholine (c).

inability of the precontracted tissues to relax when challenged with acetylcholine. The relative threshold, EC_{50}s and maximal relaxation observed for the coronary arteries with $[Mg^{2+}]_o$ ($n = 9$) were $6.4 \pm 1.2 \times 10^{-8}$ M, $3.8 \pm 0.9 \times 10^{-6}$ M, and $62.2 \pm 5.9\%$; and the relative values without $[Mg^{2+}]_o$ ($n = 9$) were $6.9 \pm 1.5 \times 10^{-6}$ M, $1.2 \pm 0.4 \times 10^{-5}$ M and $26.4 \pm 5.9\%$. Readmittance of Mg^{2+} to the bathing media restored completely the normal relaxation concentration-response curve to acetylcholine (Figure 1c).

Removal of the endothelium as described previously by rubbing (Furchgott & Zawadzki, 1980), in the presence of $[Mg^{2+}]_o$ resulted in an almost complete loss of relaxation responses to acetylcholine and often a transformation into contractile responses; removal of $[Mg^{2+}]_o$ under these conditions resulted in potentiation of contractions to acetylcholine, similar to that reported previously (Turlapaty & Altura, 1980).

Discussion Irrespective of the exact mechanism (e.g., co-factor in generation of EDRF, co-factor in generation of cyclic GMP, etc.), the results presented here clearly demonstrate that Mg^{2+} is required for the expression of coronary arterial relaxant responses to acetylcholine. The fact that the relaxation concentration-response curves to acetylcholine are profoundly depressed and shifted to higher concentrations in the absence of $[Mg^{2+}]_o$ suggests that the action of EDRF on the vascular smooth muscle cells may require Mg^{2+} for binding to the surface membrane-hormone receptors. Experiments are currently underway in our laboratory to determine whether similar findings obtain for other vasodilators which generate EDRF's. Overall, our findings, when viewed in light of previous reports which indicate that reduced $[Mg^{2+}]_o$ in the coronary vasculature environment can cause vasopasm and potentiate the contractile actions of vasoconstrictors (Turlapaty & Altura, 1980; Altura & Altura, 1985b), lends credence to the hypothesis that hypomagnesemia could produce progressive vasoconstriction, resulting in coronary arterial spasm and finally sudden-death ischaemic heart disease (Altura, 1979).

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