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Exposure of piglet coronary arterial muscle cells to low concentrations of Mg^{2+} found in blood of ischemic heart disease patients result in rapid elevation of cytosolic Ca^{2+}: Relevance to sudden infant death syndrome

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Abstract

Exposure of cultured piglet primary neonatal coronary arterial smooth muscle cells to concentrations of ionized Mg^{2+} ($[Mg^{2+}]_o$ (i.e., 0.48, 0.3, 0.15 mM) found in blood of patients presenting with ischemic heart disease and in hypoxic neonates resulted in concentration-dependent elevation in intracellular free Ca^{2+} ions ($[Ca^{2+}]_i$; the lower the $[Mg^{2+}]_o$, the higher the $[Ca^{2+}]_i$ rise. The lowest concentration of $[Mg^{2+}]_o$ tested, i.e., 0.15 mM, resulted in a clear rounding-up (i.e., contraction) of many of the coronary smooth muscle cells; reintroduction of normal 1.2 mM $[Mg^{2+}]_o$ failed to restore either normal $[Ca^{2+}]_i$ or cell shape. © 1997 Elsevier Science B.V.

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Experimental and human data suggest that magnesium plays an important role in etiology of, and death from, ischemic heart disease (Chipperfield and Chipperfield, 1978; Altura and Altura, 1990) and possibly sudden infant death syndrome (Caddell, 1978). Approximately 15 years ago, it was suggested that a progressive dietary and/or metabolic-induced loss of Mg^{2+} from the body beginning early in life, particularly in and around the coronary arteries, could lead to coronary arterial vasospasm, ischemic heart disease and sudden cardiac death (for review, see Altura and Altura, 1990). Until recently there was no way to accurately measure blood or tissue levels of ionized Mg^{2+}.

During the past 3–4 years, using specific Mg^{2+} ion-selective electrodes, we and others have demonstrated that patients with severe ischemic heart disease exhibit lowered levels of serum ionized Mg^{2+} but no change in total serum magnesium levels (Altura and Altura, 1991; Altura et al., 1994). In addition, our group has recently shown that hypoxic human neonates also demonstrate deficits in serum ionized Mg^{2+} (Marcus et al., 1997). We hypothesized that such low, defined serum $[Mg^{2+}]_o$ levels (e.g., 0.15–0.48 mM) found in high risk ischemic heart disease and hypoxic neonates might result in a rise in cytosolic intracellular free Ca^{2+} in neonatal single coronary arterial smooth muscle cells exposed to these lowered $[Mg^{2+}]_o$ levels, which might set into motion coronary vasospasm resulting in graded hypoxia and ischemia, facilitating deficits in cardiac blood flow, the end results being ischemic heart disease and/or sudden cardiac death.

Experiments were carried out on single, cultured primary coronary arterial smooth muscle cells obtained from at least 4–5 different saffan-anesthetized (12 mg/kg i.m., Glaxo or Pittman–Moore) 7–10 day old piglets using digital imaging microscopy with the fluorescent probe fura-2, using modifications of previously established methods (Zhang et al., 1996). Prior to enzymatic digestion the arteries were denuded of endothelial cells. By use of monoclonal antibodies for α-actin, and Trypan-blue exclusion, we found that 96–98% of the cells were pure vascular muscle (Zhang et al., 1996). The cells were cultured in Dulbecco's modified Eagle's medium at 37°C in a humidified atmosphere composed of 95% air/5% CO_2. The cells were exposed to 0.15, 0.3, 0.48, or 1.2 mM $[Mg^{2+}]_o$ for periods of from 15 s to 60 min. The cells were loaded with

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fura-2 (Molecular Probes, Eugene, OR, USA) by incubating them with 2 μM fura-2 acetoxymethyl ester in the culture media for 60 min under 95% air/5% CO_2. To improve loading efficiency, 0.12% pluronic F-127 (Sigma, St. Louis, MO, USA) was used in the loading media. The labeled cells were washed with modified HEPES buffer solution (in mM: NaCl 118, KCl 4.7, KH_2PO_4 1.2, HEPES 5, and glucose 10) containing the respective 0.15, 0.3, 0.48 or 1.2 mM Mg^{2+}. All ion concentrations, including Mg^{2+}, were made up precisely using ion-selective electrodes (Altura and Altura, 1991). The pH was adjusted to pH 7.4 with NaOH. Measurement of $[Ca^{2+}]_i$ was performed using a TN8500 Fluroplex Image Analyzer (Tracor Northern, Madison, WI, USA). Images of fura-2 fluorescence at 510 nm emissions were obtained with 340 and 380 nm excitation wavelengths using a silicon intensified target (SIT) camera. Fluorescence ratios ($R_{340/380}$) were obtained by dividing the 340 image by the 380 image.

An in vitro calibration method was used to calculate $[Ca^{2+}]_i$ of single piglet coronary arterial smooth muscle cells employing 0 and 2.54 mM buffered $CaCl_2$ standard solutions plus 10 mM EGTA for the maximum (R_{max}) and minimum fluorescence ratios of the 340 nm and 380 nm images. $[Ca^{2+}]_i$ was calculated according to the following equation (Zhang et al., 1996):

$$[Ca^{2+}]_i = K_d \times B \times (R - R_{min})/(R_{max} - R)$$

A K_d of 224 nM was used for the fura-2 complex (Zhang et al., 1996). B is the ratio of fluorescence intensity of fura-2 to the Ca^{2+} bound fura-2 at 380 nM. Particular care was taken to minimize photobleaching of the dye. Experiments were done in total darkness, and exposure to excitation light was less than 2 min in duration in all experiments.

Where appropriate, means ± S.E.M. were calculated and compared for statistical significance by Student's *t*-tests and analysis of variance using Scheffe's contrast test for multiple comparisons.

The data in Table 1 indicate that when the single neonatal coronary arterial smooth muscle cells are incubated for 10 min with $[Mg^{2+}]_o$ levels found in blood of ischemic heart disease patients and hypoxic neonates there is a concentration-dependent rise in $[Ca^{2+}]_i$; the lower the $[Mg^{2+}]_o$, the greater the elevation in $[Ca^{2+}]_i$. In additional experiments (not shown, $n = 20$), we found that only 15–30 s of exposure to the low $[Mg^{2+}]_o$ levels resulted in rapid concentration-dependent rises in $[Ca^{2+}]_i$ to where the levels were 50–65% of those seen at 10 min of incubation. Surprisingly, the lowest concentration of $[Mg^{2+}]_o$, i.e., 0.15 mM, resulted in a clear rounding-up of many of the coronary arterial smooth muscle cells, suggesting that these coronary cells can change shape and undergo powerful contractions in Mg^{2+}-deficient environments. Reintroduction of normal 1.2 mM $[Mg^{2+}]_o$ for periods up to 60 min failed to restore either normal $[Ca^{2+}]_i$ or normal cell shape ($n = 14$).

Table 1
Influence of low extracellular ionized Mg^{2+} on cytosolic free calcium ($[Ca^{2+}]_i$) in neonatal piglet coronary arterial smooth muscle cells

$[Mg^{2+}]_o$ (mM)	$[Ca^{2+}]_i$ (nM)
1.2	104.2 ± 0.65
0.48	128 ± 1.93 *
0.30	170 ± 1.62 *
0.15	246.6 ± 1.42 *

$n = 30$ cells. Values are means ± S.E.M.
* Significantly different from all other values ($p < 0.01$).

To our knowledge, this is the first demonstration: (1) that the low concentrations of ionized Mg^{2+}, recently found in the blood of ischemic heart disease patients (Altura and Altura, 1991; Altura et al., 1994) and hypoxic neonates (Marcus et al., 1997) can result in significant, concentration-dependent elevation in $[Ca^{2+}]_i$ in single coronary arterial smooth muscle cells and (2) low concentrations of $[Mg^{2+}]_o$ can result in rapid, irreversible contraction of single neonatal coronary arterial smooth muscle cells. These new data, thus, show that deficits in serum ionized Mg^{2+} levels are capable of producing, by a direct mechanism (in the absence of nervous elements, circulating hormones or blood), significant elevation of cytosolic free Ca^{2+} and spasm in neonatal (and probably fetal) coronary smooth muscle cells. In view of these observations, it would be important to determine if longer exposure to low $[Mg^{2+}]_o$ will result in coronary muscle cell injury and cell death, e.g., apoptosis. Progressive Ca overload in developing and adult coronary muscle cells, due to inappropriate dietary Mg intake and or errors in Mg metabolism would be expected to eventuate in severe cardiac ischemia (e.g., ischemic heart disease) and cell death.

Our findings are consistent with a vasospastic response in fetal and neonatal coronary arteries leading to coronary vascular occlusion and could help to explain the etiology of sudden infant death syndrome. A progressive vasospastic response in coronary arteries leading to coronary vascular occlusion could lead to the well-known cardiac defects seen in ischemic heart disease. The progressive rise in myocardial $[H^+]$, intracellular inorganic phosphate and lactic acid concomitant with the progressive myocardial loss in $[Mg^{2+}]_i$, phosphocreatine, lactic acid dehydrogenase and creatine phosphokinases, noted recently in intact working hearts, exposed to decreasing concentrations of $[Mg^{2+}]_o$ (Altura et al., 1993), are consistent with this hypothesis. Our findings could be used as a rational basis for pharmacotherapy with Mg^{2+} in both ischemic heart disease and sudden infant death syndrome.

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References

Altura, B.M., Altura, B.T., 1990. Magnesium and the cardiovascular system: experimental and clinical aspects updated. In: Sigel, H., Sigel A. (Eds.), Metal Ions in Biological Systems, vol. 26. Marcel Dekker, New York, NY, pp. 359–416.

Altura, B.T., Altura, B.M., 1991. Measurement of ionized magnesium in whole blood, plasma and serum with a new ion-selective electrode in healthy and diseased human subjects. Magn. Trace Elem. 10, 90–98.

Altura, B.M., Barbour, R.L., Dowd, T.L., Wu, F., Altura, B.T., Gupta, R.K., 1993. Low extracellular magnesium induces intracellular free Mg deficits, ischemia, depletion of high-energy phosphates and cardiac failure in intact working rat hearts: A ^{31}P-NMR study. Biochim. Biophys. Acta 1182, 329–332.

Altura, B.T., Bertschat, F., Jeremias, A., Ising, H., Altura, B.M., 1994. Comparative findings on serum IMg^{2+} of normal and diseased human subjects with the NOVA and KONE ISE's for Mg^{2+}. Scand. J. Clin. Lab. Invest. 54 (Suppl. 217), 77–82.

Caddell, J.L., 1978. Exploring the magnesium-deficient weanling rat as an animal model for the sudden infant death syndrome: Physical, biochemical, electrocardiographic and gross pathological changes. Pediatr. Res. 12, 1157–1166.

Chipperfield, B., Chipperfield, J.R., 1978. Differences in metal content of the heart muscle in death from ischemic heart disease. Am. Heart J. 95, 732–737.

Marcus, J.C., Valencia, G.B., Altura, B.T., Cracco, R.Q., Jean-Baptiste, D., Sinha, K., Altura, B.M., 1997. Serum ionized magnesium in premature and term infants, Pediatr. Neurol., in press.

Zhang, A., Cheng, T.P.-O., Altura, B.T., Altura, B.M., 1996. Acute cocaine results in rapid rises in intracellular free calcium concentration in canine cerebral vascular smooth muscle cells. Neurosci. Lett. 215, 57–59.