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Determination of Magnesium [Mg^{2+}] in the Rat Myocardium After Administration of Epinephrine

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ABSTRACT

This study examines the effects of intravenous epinephrine injections on the concentration of magnesium ions in the rat myocardium. Rats 35-40 days old were randomly assigned to one of three experimental groups: sham injection of 2.5 mL saline solution/kg body weight; 0.01 mg epinephrine/kg; and 0.02 mg epinephrine/kg. Analysis were performed by homogenizing the myocardial tissue with saline solution and then dialyzed for 24 hours at 4°C. Magnesium ion concentrations in the bathing solution were then determined by complexometric titration with EDTA and Eriochrome Black T indicator. A Kruskal-Wallis one-way analysis of variance revealed statistically insignificant ($p > 0.05$) difference between the groups. But, because dilution of the myocardial tissue with saline causes a shift in the ionic equilibrium of magnesium, that is quadratic in nature, the level of variation may be significant.

INTRODUCTION

Magnesium [Mg2+] is the second most abundant intracellular cation in mammalian tissue, and is the fourth most abundant of all cations. Approximately twenty-four grams are found in the bone tissue, myocardium, liver, skeletal muscles, and kidneys of the human body. However, only 1% is found within the extracellular fluid compartments of which one third is bound to plasma proteins (Reinhart, 1988). The ratio of extracellular to intracellular concentrations of the ion is estimated to be 1:19 (Weldy, 1988).

Magnesium's physiological importance is manifest in the fact that it serves as the cofactor in a myriad of enzymatic processes which include muscle contraction, mitochondrial function, and cell permeability. Recent case studies (Craddock et al., 1992) and research indicate that magnesium ions play an integral role in the maintenance of ionic membrane currents of the myocardium's excitable tissues by serving as a cofactor for the reactions that activate the sodium-potassium adenosine-triphosphatase pump (Na^+/K^+ pump) (Kirby et al., 1990; Kulick et al., 1994). Therefore, in the absence of adequate magnesium ion concentrations, the resting potential of the myocardial conduction network is hyperpolarized by the diffusion of potassium into the extracellular space, accompanied by a concomitant movement of sodium into the cell. The resulting imbalance increases cellular automaticity and excitability, which is characteristic of lethal ventricular tachyarrythmias associated with sudden cardiac death (Keren & Tzivoni, 1990).

In a study conducted of 16 post acute myocardial infarction (AMI) patients, blood serum analysis revealed a sharp increase in free fatty acid chains within the first hour of symptoms. In addition, a subtle decrease in serum magnesium ion concentrations was detected, which might be expected aa only a small percentage of total magnesium is located in the blood (Flink et al., 1981).

The aforementioned processes combination of studies lead to speculation of a correlation between the two during an AMI episode. A possible link between the two is the endogenous hormone epinephrine (Rayssiguier, 1977). In stressful situation, such as those associated with the AMI, higher brain centers stimulate the hypothalamus to release corticotropin releasing hormone (CRH), which in turn stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH) into the circulatory system. Once at the adrenal medulla, chromaffin cells are stimulated to manufacture and secrete large amounts of the catecholamines, epinephrine and norephinephrine (Shepherd, 1994).

Among epinephrine's physiological effects, via ß-cell receptor stimulation, are its positive inotropic and chronotropic properties. Also, epinephrine binds with ß-receptors found on adipocytes thereby initiating the second-messenger processes that trigger the release of high energy fatty acids for metabolism by the myocardium and other tissues (Gurr & Harwood, 1991).

The metabolism of fatty acids (via the citric acid cycle) results in the production of adenosine triphosphate (ATP) in much greater quantities than the oxidation of glucose. Because, it is estimated that 90% of all ATP molecules complex with magnesium ions in order to neutralize the negative charges of adjacent phosphate groups (Dawson, 1983), there is an influx of free magnesium ions from the extracellular space. This influx is an effort on the part of the cell to maintain a physiologic intracellular concentration of the cation (Williams, 1983; Vorman & Günther, 1993). Decreased extracellular [Mg^{2+}] results in an increased excitability within the nodal tissue and the ventricular myocardium, secondary to a shift of the threshold potential to more positive levels (Fry & Proctor, 1993).

This study examines the effects of intravenous epinephrine injection on the magnesium ion concentration in rat (*Rattus norvegious*) myocardial tissue. Magnesium levels are determined by using dialysis techniques and complexometric titrations.

MATERIALS AND METHODS

Pharmaceuticals and Reagents

Epinephrine 1:1,000 solution (Vedco, St. Joseph, MO) was diluted in sterile 0.9% sodium chloride to yield a concentration of 8 mcg/mL with fresh preparations made daily. Anesthesia was induced with diethyl ether (Millinckrodt Chemical, Paris, KY). A 0.1*M* solution of EDTA (Baker Chemical, Phillipsburg, NJ) was prepared as described by Blaedel and Knight (1954) and then diluted to 4.508 x 10^{-4}*M* by transferring a 25 mL aliquot of the original solution to a volumetric flask and filling to 250 mL. The Eriochrome Black T and pH 10 buffer solution were mixed as described by Skoog and West (1979). All reagents were prepared using cation-free water.

Animals and Treatments

Thirty white rats 35-40 days old of both sexes were obtained and subsequently housed one per cage (with free access to Purina rodent chow and tap water). The animals were randomly assigned in equal numbers to three experimental groups*: control injection of 0.9% sodium chloride, 2.5 mL/kg; low dose injection of epinephrine, 0.01 mg/kg; and high dose epinephrine injection, 0.02 mg/kg.

Anesthesia was induced by placing the rats in an etherization jar and maintained by the use of a nose cone. The external illiac vein was isolated and the appropriate injection given (t=0). Following circulation of the injected solution for four minutes, the procedure for opening the thorax was initiated by cutting of the overlying integument and making a small incision just caudal to the xiphoid process. Next, a midline cut was made by rupturing the diaphragm and proceeding through the sternum and ribs. The heart was excised (t=5minutes) by cross clamping the cardinal vessels and snipping the heart free. Finally the tissue was rinsed in 0.9% sodium chloride, placed in a plastic bag, and frozen at -5°C for later analysis.

Tissue Preparation and Analysis

After slight thawing, any remaining connective tissue was trimmed from the heart and its mass determined to the nearest 0.001 gm. The heart was minced and homogenized with a mortar and pestle in a 0.9% saline solution. The homogenate was placed in cellulose dialysis tubing (Sigma Chemical, St. Louis, MO) and the mortar and pestle rinsed twice to assure transfer. The dialysis tubing was then submerged in 50 mL of cation-free water.

Following 24 hours of dialysis at 4°C, the homogenate was discarded and the surrounding solution retained. Thirty mL aliquots of the bathing solution were transferred to test tubes and 1 mL of 0.1*M* ammonium carbonate added to precipitate calcium ions as calcium carbonate. After centrifugation (RCF=14,400) for 20 minutes, twenty five mL of the supernatant were then submitted to a clean polystyrene container where 1 mL of pH 10 buffer and 3-4 drops of Eriochrome Black T were added (Skoog & West, 1979). Finally the concentration of magnesium ions was determined by titration with a 4.508 x 10^{-4}*M* solution of EDTA until the indicator changed from red to pure blue. Because EDTA chelates magnesium ions at a 1:1 ratio, the experimental concentration of magnesium was calculated on the basis of the number of moles EDTA used.

*One animal excluded from the control group because of giving birth.

RESULTS

Intravenous injections of epinephrine appear to have only slightly influenced the levels of magnesium ions in rat (*Rattus norvegicus)* myocardial tissue (Figure 1).

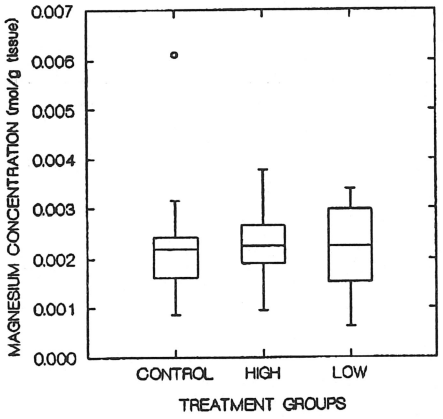

Figure 1. Comparison of magnesium levels (mol/g tissue) between the experimental groups. While there is a high degree of variation within the groups a little variation of the means ($p>0.05$), the group that received epinephrine show slightly elevated levels of magnesium ions.

These observations were in contrast to the anticipated decrease of magnesium levels, as related in the hypothesis. Specifically, the control group had a mean magnesium ion concentration of 2.018 x 10^{-3} ± 6.764 x 10^{-4} mmol/gm of tissue, whereas the low and high dose groups had levels of 2.176 x 10^{-3} ± 7.723 x 10^{-4} and 2.278 x 10^{-3} ± 7.412 x 10^{-4} respectively.

Because of the non-parametric distribution (Figures 2A, 2B, and 2C) of data within individual groups, a Kruskal-Wallis one-way analysis of variance was performed and revealed statistically insignificant correlation between the groups ($p > 0.05$). However, the possibility of epinephrine increasing the levels of magnesium was detected by the Kruskal-Wallis rank

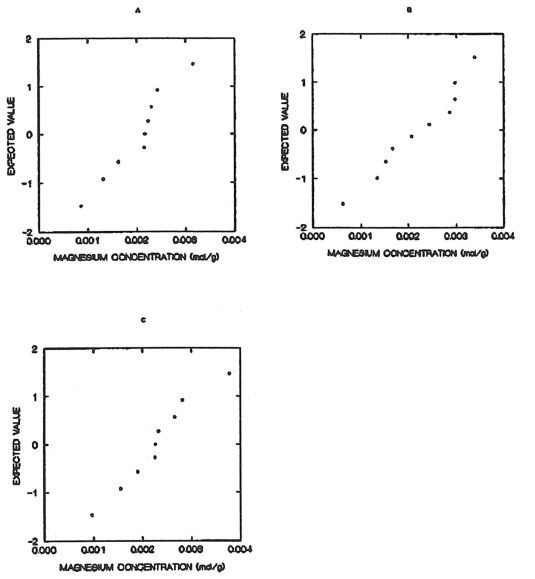

Figure 2. Probability plots of the data shows a nonparametric distribution. A - control group; B - low dose group; C - high dose group.

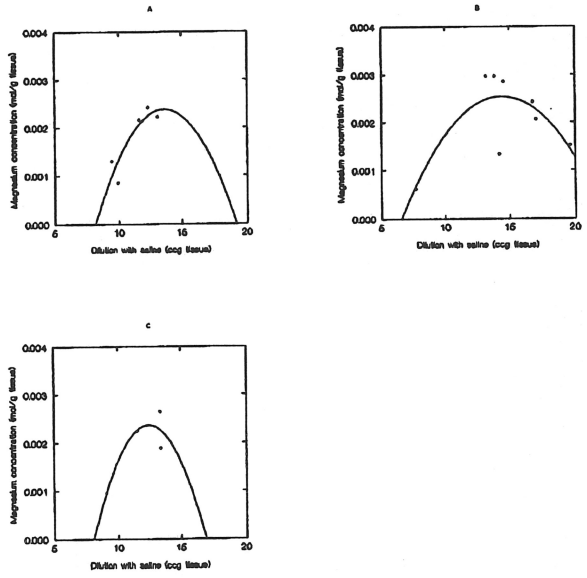

Figure 3.. Plots of the amount of saline used during homogenization versus observed levels of magnesium for males. A quadratic transformation has been applied (McGuigan et al., 1993) A - control group; B - low dose group; C - high dose group.

sum of the groups-- control, 115.0; high dose, 140.0; and low dose, 151.0.

DISCUSSION

Reconsideration of the literature and the methods employed in this study reveals information that addresses the unanticipated increase of magnesium ions, as well as supports the previously stated hypothesis.

Magnesium ions complex with proteins and other molecules, including ATP, at an equilibrium constant-- $K = [MgX]/[Mg^{2+}][X^{2-}]$. Dilution of the tissues during homogenization and dialysis alters the equilibrium between free and bound magnesium by shifting it toward the reactants (McGuigan et al., 1993). Subsequent mathematical calculations by McGuigan and colleagues show that as the dilution increases, the percentage of bound magnesium decreases logarithmically thereby resulting in an increase of detectable free magnesium concentrations. In addition, all cellular magnesium is in its free form at dilutions over 100-fold. Figures 3A, 3B, and 3C depict the experimental results transformed to fit a quadratic regression. While the vertices of the parabolas appear to be closely aligned, small variations in the dilution of the tissue sample would result in large variation of the detectable levels of magnesium by end-point titration.

The high degree of variability within all three of the experimental groups and the lack of statistically significant differences, may also be attributable to the use of EDTA and Eriochrome Black T to determine magnesium ion concentrations in tissue. While such titration methods are usually quite accurate, the subjectivity of the end point and the potential for interference from other polyvalent cations (eg. calcium and iron II) introduces a degree of uncertainty to the results.

Despite the rather statistically ambiguous results, it is fair to conclude that intravenous administration of epinephrine does exhibit an effect on the level of magnesium ions within the myocardium. But, because of the profound clinical implications of such a statement, additional, more sophisticated studies are necessary to gain better insight into the intricate cardio-physiological processes in which magnesium ions are involved.

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