Changes in Myocardial Electrolytes and Ventricular Fibrillation Threshold Induced by Alcohol Feeding in Laboratory Rats

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SUMMARY

Three groups of rats were studied in an investigation to determine the influence of alcohol on myocardial electrolytes and the ventricular fibrillation threshold (VFT) levels. Drinking water was provided ad libitum; the control group received water while the other 2 groups drank a water-ethanol solution, constituted in a ratio of 75: 25, for periods of 3 and 9 months, respectively. Two rats from each of the experimental groups, together with a control rat were killed on each experimental day. One heart was studied on the Langendorff preparation and the other used for tissue electrolyte analysis. The mean myocardial Mg^{2+} levels ($233 \pm 28 \mu g/g$ vs $148 \pm 23 \mu g/g$ and $233 \pm 28 \mu g/g$ vs $107 \pm 15 \mu g/g$; $p < 0.0001$), K^+ levels ($3260 \pm 437 \mu g/g$ vs $1779 \pm 312 \mu g/g$ and $3260 \pm 437 \mu g/g$ vs $1195 \pm 205 \mu g/g$; $p < 0.0001$) and Zn^{2+} levels ($32.7 \pm 6.8 \mu g/g$ vs $14.0 \pm 4 \mu g/g$ and $32.7 \pm 6.8 \mu g/g$ vs $4.2 \pm 3.4 \mu g/g$; $p < 0.0001$) were significantly lower in alcohol fed rats than the controls. In addition, a significant fall in the mean VFT levels (10.1 ± 1.94 mA vs 6.27 ± 2.17 mA, $p < 0.001$) was noted in rats given water: alcohol solution for 9 months. This study reveals that chronic exposure to alcohol induces a deficiency of myocardial Mg^{2+} , K^+ and Zn^{2+} , and an increase in myocardial irritability in laboratory rats.

Additional Indexing Words:

Alcohol Magnesium deficiency Potassium deficiency Zinc deficiency
Ventricular fibrillation threshold levels Langendorff preparation

MAGNESIUM, a biologically essential cation, has recently become the focus of increasing research and interest with regard to the role of its depletion in cardiovascular pathophysiology. Mg^{2+} deficiency is often overlooked as a cause of cardiac arrhythmias. Numerous studies have documented an increased incidence of ventricular tachyarrhythmias of various types,¹⁾⁻⁴⁾ including digitalis intoxication,⁵⁾ drug induced torsades de pointes,⁶⁾

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multifocal atrial tachycardia⁷⁾ and atrial fibrillation⁸⁾ with Mg^{2+} deficiency. Since alcohol is known to produce Mg^{2+} depletion in serum,⁹⁾ a study was designed to determine the effects of alcohol on myocardial electrolytes and the ventricular fibrillation threshold levels in laboratory rats.

MATERIALS AND METHODS

Sixty male rats of the University of Natal inbred Wistar strain, weighing 250–300 g, were individually ear-marked and randomly assigned to 3 groups of 20. They were housed in stainless steel cages with plastic bottoms. Drinking water was provided ad libitum. The control group received water alone, while the other 2 groups received a water-ethanol solution, constituted in a ratio of 75: 25, for periods of 3 and 9 months, respectively. All the animals were given a diet of commercial rat cubes (Epol); the mineral constituents of which appear in Table I.

After 3 months, 10 control rats and 20 alcohol-fed rats were sacrificed; the remaining control and alcohol-fed rats were sacrificed 6 months later.

Table I. Mineral Constituents of United Epol Rat Cubes

Minerals	
Calcium	10.63 g/kg feed
Phosphorus	7.56 g/kg
Magnesium	1.42 g/kg
Sodium	2.5 mg/kg
Potassium	4.43 mg/kg
Manganese	17.60 mg/kg
Manganese Sulphate	104.80 mg/kg
Zinc Sulphate	429.60 mg/kg (172 mg Zn/kg)
Copper Sulphate	838.20 mg/kg
Cobalt Sulphate	10.50 mg/kg
Ferrous Sulphate	471.50 mg/kg
Ash	4.5%

Table II. Composition of Krebs Henseleit Solution (per litre)

$NaHCO_3$	2.10 g
NaCl	6.90 g
KCl	0.35 g
KH_2PO_4	0.16 g
$MgSO_4$	0.30 g
$CaCl_2 \cdot 2H_2O$	0.36 g
Glucose	2.22 g

Rats were killed in pairs chosen at random from each group so that cardiac irritability and tissue mineral levels could be measured in matched animals. Rats were anesthetized with ether, given intravenous heparin 0.1 ml (5000 I.U.), and killed by severing the aorta at thoracotomy. In one of each pair, the heart was rapidly excised and placed in ice-cold Krebs Henseleit solution (Table II) before mounting on the Langendorff apparatus for continuous cardiac perfusion. The heart of the other rat was frozen immediately after excision and stored at -20°C to provide material for subsequent measurement of electrolytes.

Ventricular Fibrillation Threshold:

The excised heart used in the Langendorff preparation was perfused continuously with Krebs Henseleit solution, maintained at 37°C and aerated with 95% O_2 and 5% CO_2 . After a stabilization period of 15 min, measurement of heart rate began; a universal digital counter or a storage oscilloscope was used for this purpose. Coronary flow was measured by collecting the outflow of the heating chamber below the heart into a graduated measuring cylinder. The ventricular fibrillation threshold (VFT) was measured in the isolated heart using established techniques.¹⁰⁾ A bipolar electrocardiograph (ECG) was recorded continuously from two pairs of platinum electrodes, about 1 cm apart, and displayed on an oscilloscope monitoring screen. The ECG was also recorded on a tape recorder and a photosensitive paper recording machine at 25, 50 or 100 mm/sec. One electrode was attached to the metal aortic perfusion cannula. The other electrode was a very thin and flexible insulated copper wire, inserted into the epicardium of the anterior surface of the left ventricle. The site of insertion was at the midpoint between the base and the apex in a zone free of coronary artery branches. Two similar electrodes were inserted as stimulating electrodes. The cathode was placed just under the atrioventricular groove, close to the interventricular septum, while the anode was inserted at the apex of the diaphragmatic wall of the left ventricle. Ventricular fibrillation was defined as a rapid, repetitive, irregular ventricular rhythm with changing morphology which persisted for more than six cycles. To determine VFT, 200 ms pulse trains (10 ms delays, 2 ms width at 60 Hz) of increasing current intensity were applied at 30 sec delay intervals during the vulnerable period of the cardiac cycle until cardiac fibrillation occurred. Accurate positioning of stimulus and control of its sequence were achieved by an integrated control attached to a Grass stimulator. The procedure was repeated 3 times and the mean taken to represent VFT.

Measurement of Mg^{2+} , Zn^{2+} and Na^+ :

Excised hearts were placed in a 100 ml beaker containing 5 ml of 55% nitric acid. The beaker was put in a boiling water bath and digestion allowed to proceed until the heart was completely dissolved. A second beaker containing 10 ml of 60% perchloric acid was placed on a sand bath in a specialized fume cupboard. A safety visor was provided to the analyst because of the explosive nature of perchloric acid. Anti-bumping granules were added and the perchloric acid allowed to come to a boil slowly. The nitric acid solution was added drop-wise to the boiling perchloric acid and the reaction continued until dryness.^{11),12)} Distilled water was added to the cooled beaker to a volume of 10 ml and the solution stored at -20°C for subsequent analysis. All chemicals used were analytical grade. Tissue Mg^{2+} and Zn^{2+} levels were analyzed with an atomic absorption spectrophotometer (1275 Varian Model). The most common interference in air acetylene flame of Mg^{2+} analysis was overcome by the addition of lanthanum chloride (10000 $\mu\text{g/ml}$). No interference has been found in an air acetylene flame for Zn^{2+} analysis; Na^+ and K^+ were assayed by an IL 943 flame photometer using a cesium chloride, 1.5 mmol/l internal standard.

Statistical analysis of the results was carried out using the Mann Whitney Rank sum test, with a p value of 0.05 being accepted as the limit of significance for both VFT and heart tissue electrolytes.

RESULTS

After 3 and 9 months of alcohol consumption the rats appeared healthy and not visibly different from control animals. Both groups gained weight. The excised hearts appeared normal macroscopically and their weights in the acute alcohol intake group (1.08 ± 0.05 g; $n=10$) and the chronic alcohol intake group (1.12 ± 0.03 g; $n=10$) did not differ significantly from controls (0.98 ± 0.01 g; $n=10$). Detailed results appear in Tables III and IV. Concentrations of Mg^{2+} , Zn^{2+} and K^+ fell significantly in the hearts of both groups

Table III. Effect of Dietary Ethanol Supplementation on Concentrations of Several Elements ($\mu\text{g/g}$) in Rat Hearts

Group	Na^+	K^+	Mg^{2+}	Zn^{2+}
Control	819 (96)	3260 (437)	233 (28)	32.7 (6.8)
Ethanol : 3 months	1556 (375)*	1779 (312)*	148 (23)*	14.0 (4.0)*
Ethanol : 9 months	739 (398)	1195 (205)*	107 (15)*	4.2 (3.4)*

* $p < 0.0001$. $n=10$ in each group.

Results expressed as mean $\pm$ SEM.

Table IV. Effect of Dietary Ethanol Supplementation on Ventricular Fibrillation Threshold (VFT), Heart Rate (HR) and Coronary Flow (CF) of Rat Hearts

Group	VFT (mA)	HR (beats min ⁻¹)	CF (ml/min)
Control	10.10 (1.94)	204 (31)	9.70 (1.24)
Ethanol : 3 months	8.30 (4.04)	193 (18)	10.02 (1.83)
Ethanol : 9 months	6.27 (2.17)*	188 (13)	8.51 (1.53)

* $p < 0.001$. $n = 10$ in each group.
Results expressed as mean $\pm$ SEM.

of alcohol-fed rats. This change was more marked in the group which had the longest exposure to alcohol. Tissue Na^+ rose significantly after 3 months alcohol but this change was not maintained. VFT fell progressively and was significantly reduced after 9 months feeding with alcohol (Table IV). There was no change in coronary flow or heart rate in any group.

DISCUSSION

In this experiment, all cations studied (Mg^{2+} , K^+ and Zn^{2+}) were displaced down their respective electro-chemical gradients in the hearts of alcohol-fed rats. This was associated with an unequivocal fall in the ventricular fibrillation threshold. This increase in myocardial irritability was particularly marked in rats given alcohol for a period of 9 months. This result was consistent with the clinical finding that cardiac arrhythmias in patients depleted of magnesium by alcohol or furosemide respond favorably to treatment with magnesium salts.^{13),14)}

Magnesium has an important role in the maintenance of normal cardiac excitability. Movement of this ion across cell membranes may occur more rapidly in the heart muscle than in other tissues, and the heart may therefore be the first organ to be affected by magnesium loss. Magnesium is an activator of many enzymes including Na , K^+ -ATPase. The major function of this enzyme is to maintain proper ionic gradients across cell membranes which are important for controlling neural excitability and membrane transport systems. The loss of cellular magnesium results in reduction of Mg^{2+} -ATP; this may affect the Na^+ - K^+ pump thereby altering membrane potential and consequently cardiac rhythm. Numerous clinical studies have shown that magnesium therapy may be effective in the management of cardiac arrhythmias.^{15),16)}

The roles played by K^+ and Zn^{2+} and alcohol per se also require comment. Potassium is often described as a principal cause of cardiac arrhythmias. Potassium is predominantly an intracellular ion and only about

2% of the body content is in the extracellular fluid. The very low extracellular concentration of potassium ions is important for normal neuromuscular activity and cardiac action. Mg^{2+} deficiency reduces the activity of Na^+ - K^+ -ATPase, with a resultant reduction in the passage of extracellular K^+ into the cell. Intracellular K^+ deficiency is associated with a decrease in resting membrane potential, which increases myocardial excitability. This has obvious relevance to the changes in VFT observed in the course of this study.

The significance of zinc deficiency within the myocardium is speculative at present. It is uncertain whether the lack of this ion, either alone or together with magnesium and potassium deficiency, has any influence upon myocardial excitability. Zinc frequently acts as an essential co-factor to enzymatic reactions and further insight into its effects upon VFT might be obtained by prolonged studies using diets selectively deficient in zinc.

The abuse of alcohol has been suggested as a risk factor in some forms of heart disease.¹⁷⁾⁻²¹⁾ In view of the relatively long exposure to alcohol which took place in the present experiment, it is possible that significant alcohol-related changes took place in the rat myocardium. Samples were not subjected to microscopy. The findings of the present study reveal that changes in VFT and myocardial Mg^{2+} along with K^+ and Zn^{2+} might be correlated and that the VFT might be related to the intracellular activity of one or all of these substances. However, the changes in VFT were most marked in rats exposed to alcohol for the longest period. Thus, the possibility that the increase in myocardial electrical instability was a consequence of cellular damage due to alcohol per se, cannot be ruled out.

While our observations of increased myocardial electrical instability may be explicable on the basis of trace element depletion, other factors could be involved. We therefore are setting up further experiments in which alcohol-fed rats are to be given supplementary Mg^{2+} , K^+ and Zn^{2+} , to determine whether depletion of these elements is an important mechanism whereby alcohol induces morbid changes within the myocardium. This hypothesis is currently being evaluated in our laboratory.

REFERENCES

1. Loeb HS, Petras RJ, Gunnar RM, Tobin JR: Paroxysmal ventricular fibrillation in two patients with hypomagnesemia. *Circulation* **37**: 210, 1968
2. Iseri LT, Freed J, Bures AR: Magnesium deficiency and cardiac disorders. *Am J Med* **58**: 837, 1975
3. Sheehan J, White A: Diuretic-associated hypomagnesemia. *Br Med J* **285**: 1157, 1982
4. Iseri LT, Chung P, Tobis J: Magnesium therapy for intractable ventricular tachyarrhythmias in normomagnesemic patients. *West J Med* **138**: 823, 1983

5. Szekely P, Wynne NA: The effect of magnesium on cardiac arrhythmias caused by digitalis. *Clin Sci* **10**: 241, 1951
6. Tzivoni D, Keren A, Cohen AM, et al: Magnesium therapy for torsades de pointes. *Am J Cardiol* **53**: 528, 1984
7. Iseri LT, Fairshier RD, Hardemann JL, Brodsky MA: Magnesium and potassium therapy in multifocal atrial tachycardia. *Am Heart J* **110**: 789, 1985
8. De Carli C, Sprouse G, La Rosa JC: Serum magnesium levels in symptomatic atrial fibrillation and their relation to rhythm control by intravenous digoxin. *Am J Cardiol* **57**: 956, 1986
9. Brautbar N, Altura BM: Hypophosphatemia and hypomagnesemia result in cardiovascular dysfunction: Theoretical basis for alcohol induced cellular injury. *Alcoholism: Clin Exp Res* **11**: 118, 1987
10. Lubbe WF, Bricknell OL, Marzago C: Ventricular fibrillation threshold and vulnerable period in the isolated perfused rat heart. *Card Vasc Res* **9**: 613, 1975
11. Syversen TLM, Syversen GB: A simple method for acid extraction of cadmium from tissue. *Bull Environ Contam Tox* **13**: 9, 1975
12. Monk RG: A procedure for complete and controlled oxidation of organic matter by perchloric acid. *The Analyst* **87**: 64, 1962
13. Havestadt C, Ising H, Gunther T, Feldman B, Schuller HJ: Electrolytes and ventricular arrhythmias. *Magnesium* **4**: 29, 1985
14. Levin RM: The role of magnesium in cardiovascular disease. *Card Vasc Res* **10**: 37, 1985
15. Iseri LT: Magnesium in coronary artery disease. *Drugs* **28**: 150, 1984
16. Altura BM, Altura BT: Magnesium and electrolytes transport and coronary vascular tone. *Drugs* **28**: 120, 1984
17. Rubin E: Alcoholic myopathy in heart and skeletal muscle. *New Engl J Med* **301**: 28, 1979
18. Ehinger PO, Wu CF, De La Cruz JR, Weisse AB, Ahmed SS, Reagan TJ: Arrhythmias and "holiday heart" alcohol associated cardiac rhythm disorders. *Am Heart J* **95**: 555, 1978
19. Buckingham TA, Kennedy HL, Goenyian AK, Vasilomanolakis EC, Shriver KK, Sprague MK, Lyyski D: Cardiac arrhythmias in a population admitted to an acute alcohol detoxification centre. *Am Heart J* **110**: 961, 1985
20. Editorial: Dilated cardiomyopathy in Africa. *Lancet* **i**: 557, 1985
21. Editorial: Alcoholic heart disease. *Lancet* **i**: 961, 1980