ORIGINAL ARTICLE

EFFECTS OF REPEATED RESTRAINT STRESS ON SERUM ELECTROLYTES IN ETHANOL-TREATED AND WATER-TREATED RATS

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ABSTRACT

The present study was designed to evaluate the effects of simultaneous ethanol administration (10%, v/v), and restraint stress (2hrs/day for 5 day) on serum electrolytes. This restraint stress decreases serum concentration of Na^+, K^+, Ca^{2+}, phosphorus, and chloride. Ethanol treatment also decreased Na^+, K^+, Ca^{2+}, phosphorus, and chloride concentration. The decrease in Ca^{2+} and phosphorus levels was greater for ethanol-treated restrained than water - treated restrained rats. Ethanol did not affect serum Mg^{2+} while it was increased in restrained water-treated rats. Ethanol-treated restrained rats exhibited less serum Mg^{2+} than ethanol-treated unrestrained or water-treated restrained rats. Possible mechanism involved in restraint or ethanol-induced changes of electrolytes is discussed. In conclusion, the result of this study suggest that alteration of serum electrolyte caused by repeated restrained in water-treated and ethanol-treated rats could possibly occur due to an increase in sympathetic activity leading to enhanced excretion of these electrolytes.

Keywords: Ethanol, stress, electrolytes, glucose, pack cell volume.

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INTRODUCTION

Previously reported studies demonstrate that consumption of alcohol produces hormonal changes which on its turn effects absorption and metabolism of certain nutrients (Baran *et al*., 1980, Bjomeboe *et al*., 1988 and Devgun *et al*., 1981). Osteoporosis due to disturbances in calcium homeostasis may be associated with alcohol abuse (Baran *et al*., 1980., Devgun *et al*., 1981 and Ramp, 1984). The influence of ethanol administration on urinary excretion of electrolytes has been extensively studied (Carney *et al*., 1995). Peng *et al*. (1972) demonstrate that oral administration of alcohol to dogs and rats is followed within minutes by hypocalcemia and other investigators have confirmed these findings (Money *et al.,* 1989 and 1990). In contrast, studies on human showed that moderate doses of alcohol did not decrease serum calcium concentration (Ljunghall *et al*., 1985 and William *et al*., 1978). Acute and chronic ethanol administration alters the renal handling of magnesium, calcium and zinc (Kalbfleisch *et al*., 1963 and Sargent *et al*., 1974). Biochemical investigation performed to explore the mechanisms underlying stress-induced elevation in blood pressure has been shown to increase the catecholamines, rennin- angiotensin aldosterone system, cortisol, ACTH and vasopressin in various stress situation (Carlsson *et al*., 1989 and Reis and Ledoux, 1987). Restraint stress has been shown to decrease the concentration of Na^+, K^+ and Ca^{2+} in serum (Mehboob *et al*., 1996).

The present study is aimed to evaluate the simultaneous effect of ethanol administration and restrained stress on serum electrolytes in rats.

MATERIALS AND METHODS

Animals

Locally bred male Albino-Wistar rats (weighing 180-240 grams) were housed individually under 12-hour light/dark cycle with free access to cubes of standard rodent diet and tap water for at least 3 days before experiment.

Experimental protocol

Animals were randomly assigned to non- ethanol-treated and ethanol- treated groups. Ethanol was added in the drinking water of ethanol- treated group for one week, in concentrations of (10%, v/v), whereas control group received tap water. These two groups were further divided to unrestrained and restrained groups. Animals of the restrained group were immobilized, as describe earlier (Haleem *et al*., 1988) for 2h on each of the 5 following days (between 9:30 a.m. to 11:30 a.m.). The animals of unrestrained groups were left in their cages during this period. Animals were killed by cervical dislocation. Blood samples were collected and serum separated by centrifugation was analyzed for serum Na^+, K^+ and Ca^{2+} by Corning - 400 flamephotometer. Mg^{2+} was estimated by titan yellow method (Hallry and Skypeck, 1964). Phosphorus was determined by Gomorri method (Gomorri, 1942) and serum chloride by titration method (Schales and Schales, 1941). Serum glucose was determined by orthotoluidine method (Hultman, 1959) and pack cell volume was determined by centrifuging blood in a Hawksley microhematocrit centrifuge.

RESULTS

Effects on serum Na^+, K^+, Ca^{2+} and Mg^{2+}

The results are given in fig. 1 (ABCD). Analysis by two-way ANOVA showed significant effect of ethanol on serum Na^+ ($F= 9.31$, df 1, 20, $p < 0.01$), K^+ ($F= 31.32$, df 1, 20, $p < 0.01$), Ca^{2+} ($F= 48.57$, df 1, 20, $p < 0.01$) and Mg^{2+} ($F= 14.97$, df 1, 20, $p < 0.01$). The stress effects were significant for Na^+ ($F= 6.93$, df 1, 20, $p < 0.01$), K^+ ($F= 11.92$, df 1, 20, $p < 0.01$), Ca^{2+} ($F= 13.27$, df 1, 20, $p < 0.01$) and non- significant for Mg^{2+} ($F= 2.83$, df 1, 20, $p > 0.05$). Interaction of ethanol and stress was significant for only K^+ ($F= 4.42$, df 1, 20, $p > 0.05$) and insignificant for Na^+ ($F= 0.26$, df 1, 20, $p > 0.05$), Mg^{2+} ($F= 0.06$, df 1, 20, $p < 0.01$) and Ca^{2+} ($F=0.3$, df 1, 20, $p > 0.05$).

Individual comparison by Newman-Keuls test revealed restrained stress decreased serum Na^+ ($P<0.05$), K^+ ($P<0.01$) and Ca^{2+} ($P<0.05$) and increased Mg^{2+} ($P<0.05$) concentration in water- treated rats. Effects of restraint which has decreased the Na^+ and Mg^{2+} was found greater in ethanol - treated than water - treated rats. Ethanol - treatment also decreased Na^+, K^+ and Ca^{2+} concentration without producing an effect on Mg^{2+} levels.

Effects on serum phosphorus, chloride, glucose and pack cell volume

Effects of immobilization stress (2h/day for 5 days) on ethanol induced changes of serum phosphorus, chloride, glucose and pack cell volume are shown in fig. 2 (ABCD). Analysis by Two-way ANOVA showed significant effects of ethanol on serum phosphorus ($F= 14.19$, df 1, 20, $p < 0.01$), Cl^- ($F= 13.38$, df 1, 20, $p < 0.01$), glucose ($F= 11.74$, df1, 20, $P<0.01$) and insignificant effect ($F= 2.79$, df 1, 20, $p >< 0.05$) on pack cell volume. The stress effect were significant for serum phosphorus ($F= 42.83$, df 1, 20, $p < 0.01$), chloride ($F= 16.76$, df 1, 20, $p < 0.01$), glucose ($F= 10.21$, df1, 20, $P<0.01$) and pack cell volume ($F= 5.35$, df 1, 20, $p < 0.05$). Interaction between ethanol and stress was significant foe glucose ($F= 5.97$, df1, 20, $P<0.05$) and chloride ($F= 9.78$, df 1, 20, $p < 0.01$) and insignificant for phosphorus ($F= 1.66$, df 1, 20, $p > 0.05$) and pack cell volume ($F= 0.14$, df 1, 20, $p > 0.05$).

Follow-up comparison by Newman-Keuls test indicated that restrainet stress decreased serum phosphorus ($P<0.01$) and chloride ($P<0.01$) levels in water-treated

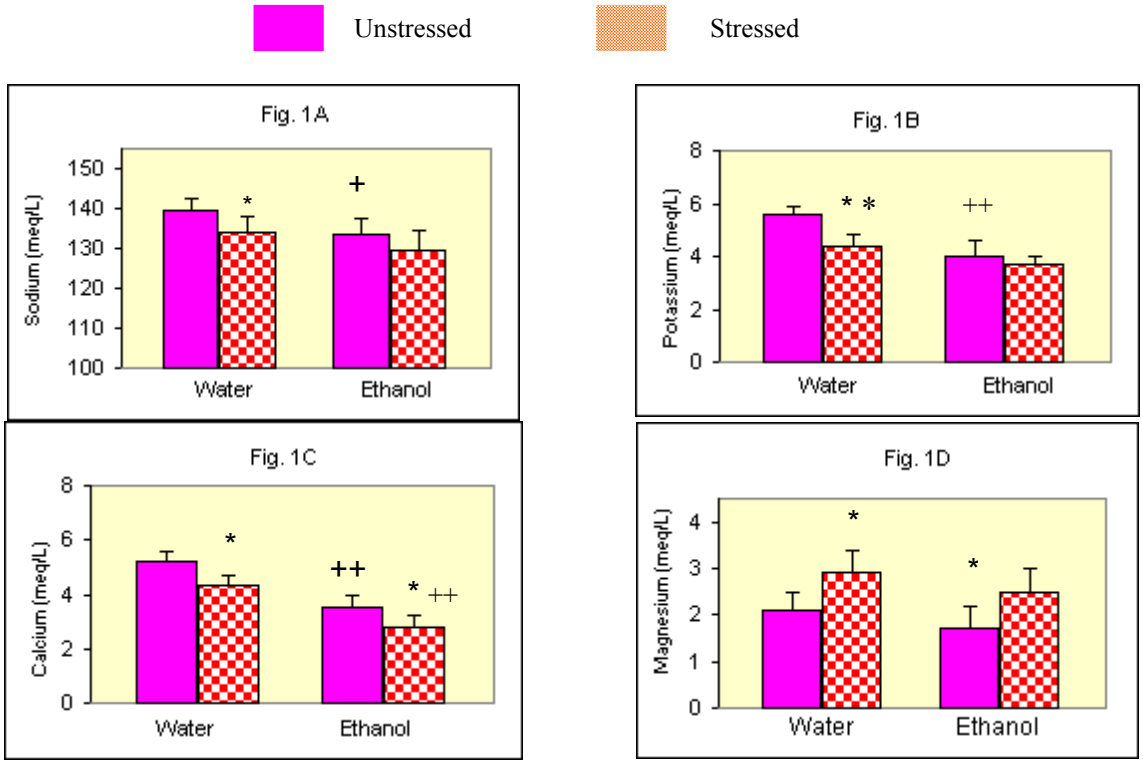

Fig. 1: Effects of restraint stress on serum Na^+, K^+, Ca^{2+} and Mg^{2+} in non. Ethanol-treated and ethanol - treated rats. Values are means ± SD (n = 6) of unrestrained or restrained rats. Significant differences by Newmann-Keuls test. *P<0.05, **P<0.01 from respective unrestrained rats, $^+$P<0.05, $^{++}$P<0.01 from respective water- treated rats following 2-way ANOVA.

rats. Decreases in glucose and pack cell volume were not statistically significant. Ethanol-treated restrained rats exhibited decreased serum phosphorus levels than their unrestrained counterparts, the fall in glucose, chloride and pack cell volume was statistically insignificant. The fall in phosphorus were greater in ethanol-treated restrained than water-treated restrained rats. Ethanol treatment decreased only chloride levels while phosphorus, glucose and pack cell volume did not alter.

DISCUSSION

Possible explanation for the observed decreased in Na^+ concentration following ethanol administration is inhibition of antidiuretic hormone release resulting in a decrease of the osmo- sodium receptors and enhanced urine formation (Eisenhoffer and Johnson, 1982). It is often suggested that atrial natriuretic peptide (ANP) could have an important role in alcohol- induced diuresis (Colantonio *et al*., 1991). Acute ethanol intake causes an increase in the plasma ANP (Bezzegh *et al*., 1991). Stress has been also reported to increase the release of ANP (Horky *et al*., 1985). Higher levels of ANP suppress arginine vasopressin (AVP) release from the supraoptic nucleus (Clark *et al*., 1991). In fact, at physiological concentration, ANP causes a large inhibition of vasopressin - stimulated osmotic water permeability in the rat terminal inner medullary-collecting duct (Nonouguchi *et al*., 1988) causing diuresis and natriuresis.

Hypokalemia effects of restraint stress and also of ethanol are explainable in term of increase in plasma concentration of catecholamines (Zgombick *et al*., 1986) as epinephrine's action via β-2 receptor mechanism could promote the movement of potassium from extracellular fluid to intracellular compartment to decrease serum levels of K^+ (Clausen and Flatman, 1980).

Ethanol-induced hypocalcaemia has been observed in dogs and rats (Mahboob and Haleem, 1988, Peng *et al*., 1972). Parathyroid hormone (PTH), is an important regulator of blood calcium levels. It acts on kidneys to promote reabsorption of Ca^{2+} from the ultrafilterates and on bones to release Ca into the circulation. It also stimulates production of active vitamin D metabolite, which facilitates gut Ca^{2+} absorption (Fujii *et al*., 1988). Ethanol administration has been shown to depress PTH secretion (Turner *et al*., 2001 and Laitinen *et al*., 1991), which can also lead to increase Ca^{2+} excretion (Massry *et al*., 1968). Ethanol-induced fall in PTH secretion may be responsible for a fall in serum Ca^{2+} observed in the present study (fig. 1C).

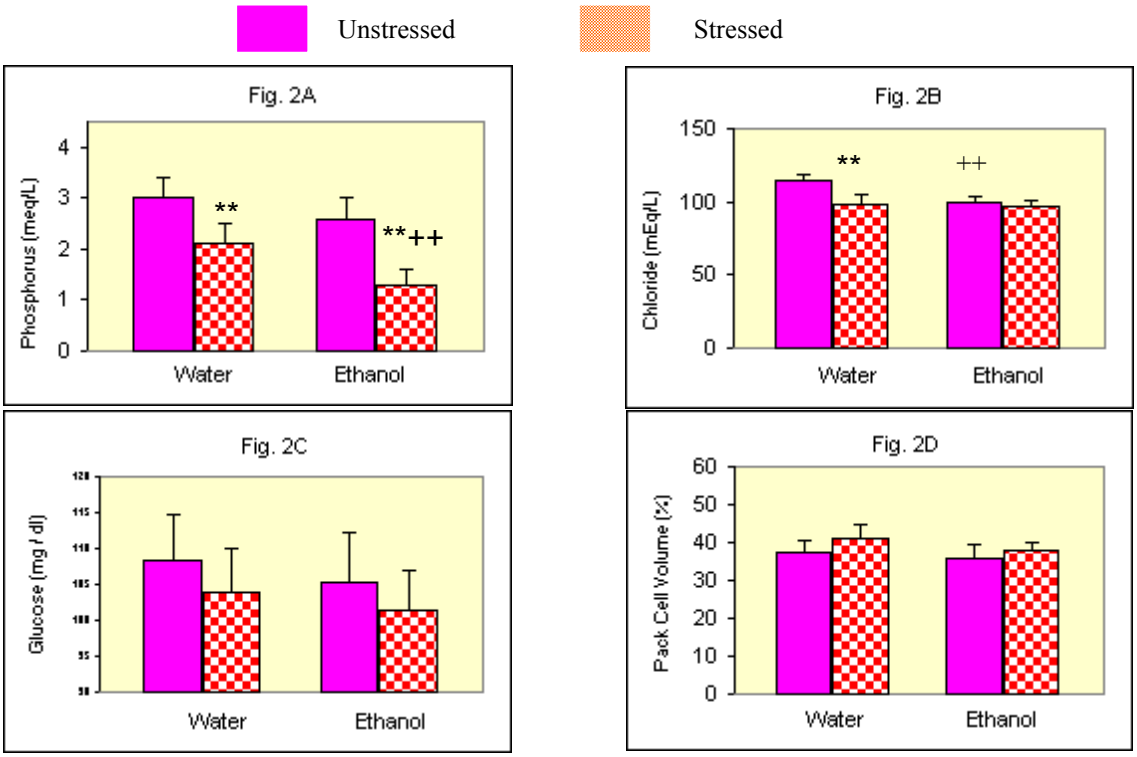

Fig. 2: Effects of restraint stress on serum phosphorus, chloride, gtlucose and packed cell volume in non-ethanol-treated and ethanol-treated rats. Values are means ± SD (n=6) of unrestrained or restrained rats. Significant differences by Newmann-Keuls test. *P<0.05, **P<0.01 from respective unrestrained rats, $^{+}$P<0.05, $^{++}$P<0.01 from respective water-treated rats following 2-way ANOVA.

Stress stimulates hypothalamic - pituitary axis to increase plasma levels of glucocorticoids including cortisol (Kant *et al*., 1983). Elevated levels of cortisol increase Ca^{2+} excretion (Massry *et al*., 1968 and Cicero, 1981). Restraint stress-induced decreases of Ca^{2+} as observed in the present study (fig. 1) are therefore atleast partly also explainable in terms of higher level of ethanol. However, plasma levels of cortisol were not determined in the present study.

Hypermagnesemia observed following restraint in water-treated rats is consistent with previous reports (Mahboob and Haleem, 1988). In addition, the present study shows that restraint given to ethanol - treated rats also decreases serum Mg^{2+} concentration. Hypermagnesemia has been reported following ethanol injection (Mahboob and Haleem, 1988). In the present study where ethanol was added in the drinking water, the changes observed in serum Mg^{2+} concentration were statistically insignificant than non - ethanol treated rats.

In conclusion the present study shows a decrease in serum electrolytes such as of Na^{+}, K^{+}, Ca^{2+} and Mg^{2+} following restraint, ethanol and restraint plus ethanol together. These decreases could possibly occur due to an increase in sympathetic activity leading to enhance excretion of these electrolytes. Decreases of phosphorus and chloride concentration may occur to compensate the loss of cations. To find a possible mechanism involved in stress or ethanol-induced electrolyte changes requires more investigation.

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