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Effect of Ethanol on Regulation of (Na + K)–Adenosine Triphosphatase by Aldosterone and Dexamethasone in Cultured Renal Papillary Collecting Duct Cells

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Chronic ethanol exposure causes alterations in biologic membranes of different cell types. (Na + K)–adenosine triphosphatase (ATPase), a membrane-bound enzyme inhibited by the acute presence of ethanol, increases its activity in rat kidney after chronic ethanol consumption. The aim of this investigation was to evaluate the effect of ethanol on the modulation of (Na + K)-ATPase by glucocorticoids and mineralocorticoids in renal papillary collecting duct cells. Cultured renal papillary collecting duct cells were exposed to a medium containing 150 m*M* ethanol plus either 100 n*M* aldosterone or 10 n*M* dexamethasone. Control groups were cultured in the absence of ethanol and/or the hormones. Mg^{2+}-ATPase was used as control enzyme. The activity of ATPases was measured by ATP hydrolysis. Ethanol increased the activities of (Na + K)-ATPase and Mg^{2+}-ATPase in 29 and 33% of controls, respectively; only (Na + K)-ATPase activity was elevated in the presence of aldosterone or dexamethasone, whereas Mg^{2+}-ATPase was unaltered by these hormones. The effects of aldosterone and dexamethasone on (Na + K)-ATPase activity were augmented by ethanol in 50 and 19% of controls, respectively. These results suggest that ethanol treatment enhances the upregulation of (Na + K)-ATPase activity by both aldosterone and dexamethasone, in cultured renal papillary collecting duct cells.

Key Words: (Na + K)–adenosine triphosphatase; kidney; ethanol; aldosterone; dexamethasone.

Introduction

A considerable body of evidence has implicated ethanol consumption in the morphologic and functional damage found in organs such as the liver and central nervous system; however, few studies have been carried out on kidney alterations *(1,2)*. It is well recognized that the pharmacologic effects of ethanol are partly related to its interaction with the membrane phospholipids. Thus, ethanol may quickly penetrate the lipid bilayer, thereby affecting the structural and physicochemical properties of biologic membranes *(3)*. In acute administration, ethanol causes a disorder in the bilayer, enhancing membrane fluidification *(4)*, whereas in chronic exposure, membrane reaches resistance to this ethanol-induced disordering effect *(5)*. As a consequence, the membrane-bound enzymes are frequently inhibited by acute ethanol in vitro. (Na + K)–adenosine triphosphatase (ATPase) follows this pattern *(6)*, but chronic ethanol exposure causes an enhancement of this activity in vivo in brain *(7)*, liver *(8)*, skeletal muscle *(9,10)*, and erythrocytes *(11)*. We also have found that chronic ethanol feeding generates increased (Na + K)-ATPase activity in renal cortex and outer medulla of adult *(6,12)* and growing rats *(13)*. This upregulation of (Na + K)-ATPase has also been found in cultured renal papillary collecting duct cells chronically exposed to ethanol *(14)* and may be involved in the antinatriuretic effect of ethanol *(15,16)*. Nevertheless, the mechanism of this enzyme activation is not well understood. (Na + K)-ATPase activity is under the control of several hormones *(17)*, whose effects could be modulated by ethanol, but studies on the effects of ethanol on the hormonal control of this enzyme in tubular epithelial cells are still lacking. The aim of the present investigation was to determine the effect of chronic ethanol exposure on the regulation of renal (Na + K)-ATPase activity by aldosterone and dexamethasone in primary cultures of rat renal papillary collecting duct cells.

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Results

Effect of Chronic Ethanol Exposure on (Na + K)-ATPase and Mg^{2+}-ATPase Activities

After chronic ethanol treatment, the activity of (Na + K)-ATPase (micromoles of Pi per milligram of protein per hour) in cultured renal papillary collecting duct cells was 22.1 ± 1.2 (n = 16), whereas the control value without ethanol was 17.0 ± 0.8 (n = 15) (Fig. 1). Thus, the presence of ethanol caused a 29% increase in enzyme activity. The activity of

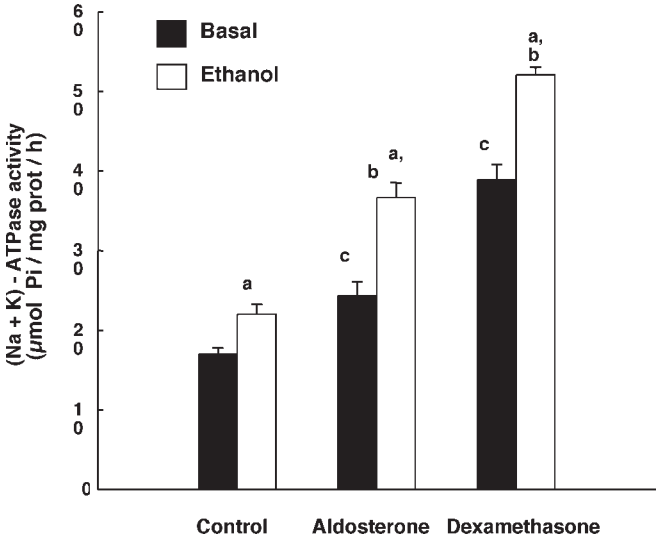

Fig. 1. Effects of ethanol on regulation of (Na + K)-ATPase activity by aldosterone and dexamethasone in cultured renal papillary collecting duct cells. Results are means of 15 to 16 values ± SEM. Statistically significant differences, at $p < 0.05$, are indicated by superscript letters: [a]vs basal in each treatment; [b]vs control-ethanol; [c]vs control-basal.

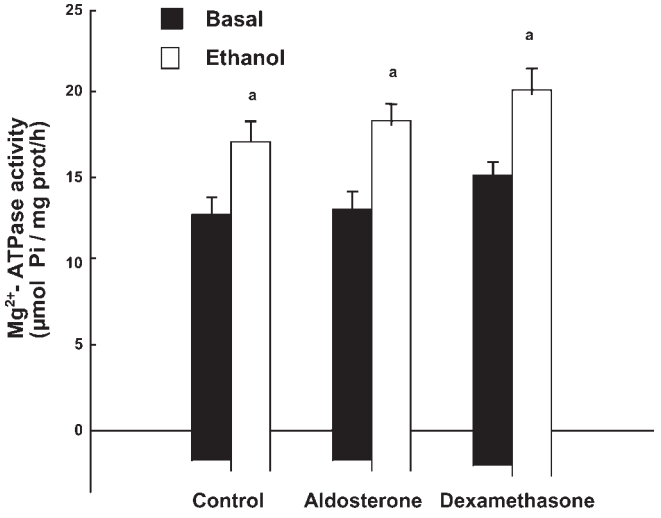

Fig. 2. Effects of ethanol on regulation of Mg^{2+}-ATPase activity by aldosterone and dexamethasone in cultured renal papillary collecting duct cells. Results are means of 15 to 16 values ± SEM. Statistically significant differences, at $p < 0.05$, are indicated by superscript letters: [a]vs basal in each treatment.

Mg^{2+}-ATPase was 17.1 ± 1.0 and 12.8 ± 0.9, for ethanol-treated and control cultures, respectively, representing a 33% increase. The differences were significant for both enzymes ($p < 0.05$).

Effect of Aldosterone and Dexamethasone on (Na + K)-ATPase and Mg^{2+}-ATPase Activities

The effects of aldosterone and dexamethasone on the activity of (Na + K)-ATPase are shown in Fig. 1 and for Mg^{2+}-ATPase in Fig. 2. (Na + K)-ATPase activity was upregulated in the presence of either aldosterone or dexamethasone, since it was increased by 41 and 160% of control, respectively. In the presence of ethanol, aldosterone and dexamethasone caused a response further enhanced by 50 and 19%, respectively ($p < 0.05$). The activity of Mg^{2+}-ATPase did not show a further increase by the presence of the hormones plus ethanol.

Discussion

The purpose of the present study was to investigate the effects of chronic ethanol exposure on the regulation of renal (Na + K)-ATPase activity by glucocorticoids and mineralocorticoids. The experimental design reported here has been previously applied to PC12 cells. We found that chronic ethanol exposure increased the activity of both (Na + K)-ATPase and Mg^{2+}-ATPase, but only the first was upregulated by aldosterone and dexamethasone. These results are consistent with other data showing a dose-dependent response to ethanol of these ATPases of cultured PC12 cells *(18)*. Oberdoerster et al. *(19)* showed a maximal increase in enzyme activity at 150 m*M* ethanol, the same concentration used in the present study, which does not significantly influence cell viability. This pattern of ethanol-induced ATPase upregulation has also been reported in other cell types, such as erythrocytes of alcoholic patients showing a marked enzyme activation that was reversed after 3 mo of abstinence *(20)*. Previously, we found a similar pattern of response in the kidney of the rat, explaining the mechanism of upregulation by an increased number of catalytic units, but not on the basis of an augmentation of their intrinsic activity *(21)*. This enzyme response was characterized by changes in the kinetic parameters of renal (Na + K)-ATPase, as assessed by increased V_{max} for substrates K^+ and ATP, whereas K_m and Hill coefficient were unaltered *(6)*.

The complex regulation of (Na + K)-ATPase activity has made elucidating the mechanism of the response of target cells to ethanol in vivo difficult *(22)*. The activity of renal (Na + K)-ATPase is influenced by other effects including those provided by hemodynamic, neural, humoral, and local factors. Therefore, it is not feasible to extrapolate the results obtained by this model to a polarized epithelium of whole kidney in vivo. Nevertheless, our study was carried out in cultured renal papillary collecting duct cells, an experimental design that has the advantage of allowing the possibility of analyzing, separately, the contribution of extracellular factors, such as ethanol and/or the hormones, under controlled conditions. Thus, these experimental conditions may represent a better way to ascribe to hormonal and ethanol

effects the changes observed in the activity of (Na + K)-ATPase.

The relevance of the endocrine effects controlling the activity of renal (Na + K)-ATPase has been widely accepted *(17)*. Since the upregulation of this enzyme activity by ethanol has been attributed to an increased number of catalytic units, the contribution of steroidal and thyroid hormones had to be considered. To ascertain this mediation, we measured the plasma levels of triiodothyronine, levorotatory thyroxine, glucocorticoids, and mineralocorticoids in rats following chronic ethanol consumption. Nevertheless, unexpectedly, we could not demonstrate changes in the plasma levels of these hormones, which would explain the increased activity of renal (Na + K)-ATPase *(23–25)*. Therefore, it was necessary to assess the possible modification of the response of the target cells to the hormonal regulation. The renal papillary collecting duct cells, used in our study, constitute a type of cells easily isolated from the kidney that are sensitive to both aldosterone and glucocorticoids. As expected, in our study, the presence of aldosterone and dexamethasone caused a selective increase in the (Na + K)-ATPase activity in the cultured epithelial tubular cells, without change in the activity of Mg^{2+}-ATPase. On the other hand, the presence of ethanol exacerbated these hormonal effects (Fig. 1), a finding that could help to explain the previously reported data showing upregulation of renal (Na + K)-ATPase by ethanol in vivo, in the absence of changes in plasma levels of aldosterone and corticosterone. The mechanism whereby this effect is exerted has to be elucidated. The effect of ethanol exposure on the transduction signaling pathways occurring during the hormonal response of these epithelial cells should be investigated. In addition, the possible effect of other hormones, not evaluated yet, in this experimental setting should not be ruled out.

Apart from the hemodynamic, neural, local, and endocrine regulatory events accounting for the ethanol-induced upregulation of (Na + K)-ATPase, a role of oxidative stress, as suggested by the demonstration that an iron chelator impedes this effect caused by ethanol in the enzyme of erythrocytes, cannot be discarded *(11)*. It has been reported that ethanol increases lipid peroxidation in the membranes of these cells, thereby raising the possibility of reducing membrane fluidity by molecular arrangement *(26)*. Homologous experiments in papillary collecting duct cells could be carried out to corroborate whether this phenomenon is involved in the enhancement of renal (Na + K)-ATPase activity caused by chronic ethanol exposure. Although the physiologic or pathophysiologic relevance of these findings remains to be determined, it deserves special mention that this response of renal (Na + K)-ATPase is associated with the occurrence of a drop in the fractional excretion of sodium, without changes in the glomerular filtration rate, supporting its physiologic implication for the renal tubular sodium reabsorption in both rats *(16)* and humans *(27)*.

In summary, the results of the present study suggest that chronic ethanol exposure induces upregulation of (Na + K)-ATPase of renal papillary collecting duct cells, but it also causes an exacerbated response of these epithelial cells to the enzyme modulation by both mineralocorticoids and glucocorticoids.

Materials and Methods

Culture Media, Hormones, and Reagents

The culture media, hormones, and biochemical reagents were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were obtained from commercial sources and were of the highest purity available. Rat pellets were from Champion SA (Santiago, Chile).

Animals

Male adult Wistar rats (*Rattus norvegicus*), weighing 220–250 g (ICBM, Programa de Farmacología Molecular y Clínica, Facultad de Medicina, Universidad de Chile, Santiago, Chile), were given free access to pellet chow (Champion SA) and water before the experiments. A group showing normal plasma values for creatinine and urea nitrogen was chosen. The animals were anesthetized with ethyl ether before being killed by decapitation, and the kidneys were weighed and homogenized within 30 min after death. The rats were treated according to internationally accepted rules of ethics.

Purification of Rat Papillary Collecting Duct Cells

The papillae were cut out and immediately placed in an ice-cold HEPES buffer solution, containing 118 m*M* NaCl, 16 m*M* HEPES, 17 m*M* Na-HEPES, 14 m*M* glucose, 3.2 m*M* KCl, 2.5 m*M* $CaCl_2$, 1.8 m*M* $MgSO_4$, and 1.8 m*M* KH_2PO_4 at pH = 7.4. Renal papillary collecting duct cells were isolated from renal tissue according to Stokes et al. *(28)*. Cell viability was >90%, as estimated by trypan stain exclusion.

Primary Cultures of Renal Papillary Collecting Duct Cells

Primary cultures were obtained from renal papillary collecting duct cells in sterile conditions. To prevent bacterial contamination, phosphate-buffered saline buffer (pH 7.20) containing gentamicin (50 μg/mL) (Laboratorio Chile, Santiago, Chile) was used. The final pellet was suspended in the culture medium (Dulbecco's Modified Eagle's medium, HAM F12) containing 10% fetal bovine serum. Cells were seeded in multiwell plates (Costar, Cambridge, MA) and were grown under 5% CO_2 atmosphere reaching a density of 200,000 cells/cm^2 in each 1-mL plate.

Ethanol and Hormone Treatments of Primary Cultures

Chronic ethanol treatment (ethanol-treated cultures) was carried out during the last 4 d by the addition of 150 m*M* ethanol to the culture medium, a treatment previously reported

that did not significantly influence cell viability *(19,29)*. To evaluate the contribution of ethanol to the endocrine regulation of (Na + K)-ATPase of renal papillary collecting duct cells, primary cultures of these epithelial cells were cultured in the presence and absence of either 100 n*M* aldosterone for 72 h *(24)* or 10 n*M* dexamethasone for 48 h *(30)*. Then the cells were loosened from the plates after incubating for 30 min at 37ºC with 0.05% (w/v) trypsin. Finally, the cells were washed once in 20 mL of Dulbecco's phosphate-buffered and serum-free medium and centrifuged at 50*g*. The pellet was suspended and homogenized in a freshly prepared buffer solution (pH 6.80) containing 0.25 *M* sucrose, 30 m*M* histidine, 5 m*M* disodium EDTA, and 2.4 m*M* sodium deoxycholate.

Enzyme Assays

The activities of (Na + K)-ATPase and Mg^{2+}-ATPase were measured by the method of Katz and Epstein *(31)*. Aliquots (10 µL) of a whole homogenate of the primary cultures were incubated in 500 µL of a medium containing 100 m*M* NaCl, 20 m*M* KCl, 6 m*M* $MgCl_2$, 6 m*M* ATP disodium salt (vanadium free), and 10 m*M* imidazole at pH 7.80. The final protein concentration was from 0.02 to 0.03 mg/mL. All components of the incubation mixture were preincubated at 37ºC for 1 min, and the reaction was started by the addition of ATP and stopped 15 min later with 1 mL of ice-cold 25% (w/v) trichloroacetic acid. Inorganic phosphate in the supernatant was measured by the method of Taussky and Shorr *(32)*. (Na + K)-ATPase activity was calculated from the difference in the amount of inorganic phosphate released in the presence and in the absence of K^+ in the incubation medium. Mg^{2+}-ATPase activity was calculated from the difference between the amount of inorganic phosphate released in the absence of K^+ and that found at zero time. Protein content was measured by the method of Lowry et al. *(33)*, using crystalline bovine serum albumin as standard. The specific activities were expressed as micromoles of inorganic phosphate released per milligram of protein per hour.

Statistical Analyses

Results are expressed as the mean values ± SEM. The sources of variations were assessed by one-way analysis of variance, and comparisons for individual differences between groups were done using the Scheffé test. Differences were considered statistically significant at $p < 0.05$.

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