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EFFECT OF ETHANOL AND ACETALDEHYDE ON THE $(Na^+ + K^+)$-ACTIVATED ADENOSINE TRIPHOSPHATASE ACTIVITY OF CARDIAC PLASMA MEMBRANES*

JOSEPH W. WILLIAMS, MICHIHIKO TADA, ARNOLD M. KATZ and EMANUEL RUBIN

Department of Pathology and Division of Cardiology, Department of Medicine, Mount Sinai School of Medicine of the City University of New York, New York, N.Y., U.S.A.

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Abstract—Ethanol and acetaldehyde inhibited the $(Na^+ + K^+)$-activated ATPase activity of plasma membranes prepared from the guinea-pig heart. The degree of inhibition was dose-dependent and antagonized by the K^+ concentration in the reaction mixture. The inhibition is not attributable to increase in osmolality. The presence of ethanol or acetaldehyde in the reaction mixture was necessary for the inhibitory effect. Plasma membranes treated with ethanol or acetaldehyde and subsequently washed showed no impairment of $(Na^+ + K^+)$-activated ATPase activity. Prolonged exposure of the plasma membranes to a low concentration of ethanol was ineffective in increasing the inhibition of ATPase activity.

Cardiomyopathy associated with chronic alcoholism is an established clinical entity [1–3], which is independent of nutritional factors. Moreover, acute ethanol administration produces a negative inotropic effect in experimental animals [4–6] and in man [6, 7]. At the cellular level, ethanol influences transport of Na^+, K^+ and amino acids [8], and inhibits the $(Na^+ + K^+)$-activated ATPase of brain tissue *in vitro* [9–13]. We therefore examined the effect of ethanol and its metabolite, acetaldehyde, on the $(Na^+ + K^+)$-activated ATPase activity of a morphologically recognizable preparation of myocardial plasma membranes. These isolated membranes contain elements derived from the Na^+ transport system, one of the key regulatory factors in the control of myocardial contractility.

MATERIALS AND METHODS

Plasma membranes of guinea-pig hearts were prepared by the method of Tada *et al.* [14]. English short-hair strain guinea-pigs weighing 500–600 g were killed by a sharp blow to the head. The hearts were promptly excised, washed and immersed in ice-cold 0·25 mM EDTA†–2·5 mM imidazole (pH 7·5). After removal of the atria and the great vessels, the ventricles were sliced and homogenized in 10 vol of 0·25 mM EDTA–2·5 mM imidazole (pH 7·5) for 1–2 min at high speed in a Virtis, model 45, Omnimixer. The supernatant, containing segments of one to four cells (determined by phase contrast microscopy) was decanted and set aside. The remaining pieces of myocardium in the pellet were again homogenized and the supernatant was added to the first supernatant. This procedure was repeated six times. The pooled supernatant was filtered through four layers of gauze and centrifuged at 4° for 30 min at 1600 *g*. The pellet was washed twice with ice-cold 10% sucrose–0·2 mM EGTA–10 mM Tris–HCl (pH 7·5), after which the final pellet was extracted overnight at 4° in 1 M KCl–40% sucrose–2·0 mM EDTA–10 mM Tris–HCl (pH 7·0). The extracted membranes were collected by centrifugation, the supernatant was discarded and the pellet washed twice with 25 vol of ice-cold 5 mM Tris–HCl (pH 7·5). The final pellet was suspended in 40% sucrose–0·2 mM EGTA–10 mM Tris–HCl (pH 7·5). Protein concentration was determined by the biuret reaction with bovine serum albumin as the standard.

The $(Na^+ + K^+)$-activated ATPase activity of the plasma membranes was determined in triplicate. The standard reaction mixture contained 0·2–0·3 mg/ml of membrane protein, 5 mM MgATP, 10^{-8} M Ca^{2+} (Ca–EGTA buffer [15]) and 40 mM imidazole buffer (pH 6·8) with either 120 mM NaCl, 100 mM NaCl + 20 mM KCl or 115 mM NaCl + 5 mM KCl. The reaction was started by the addition of MgATP and carried out for 10 min at 37°. Ethanol or acetaldehyde was added to stoppered tubes 3 min prior to the addition of MgATP. Reactions were terminated by addition of an equal volume of 20% trichloroacetic acid, and the tubes transferred to ice. The amount of Pi liberated was determined by the method of Taussky and Shorr [16]. The difference between the ATPase activity in the presence of both Na^+ and K^+ and that seen in the presence of Na^+ alone is defined as the $(Na^+ + K^+)$-activated ATPase. This corresponds closely to the ouabain-sensitive ATPase [14].

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† Abbreviations: EDTA = ethylenediamine tetraacetic acid; EGTA = 1,2-*bis*-(2-dicarboxymethyl amino ethoxy) ethane; ATP = adenosine triphosphate; ATPase = adenosine triphosphatase; Tris = tris (hydroxymethyl) aminomethane.

Pretreatment of plasma membranes with ethanol or acetaldehyde was performed as follows. Procedure A: Plasma membranes (6 mg/ml) were incubated at 25° in a 40% sucrose solution containing 0·1 to 0·5 M ethanol, 0·18 mM EGTA and 9 mM Tris–HCl (pH 7·5). Control preparations were simultaneously incubated with buffer instead of ethanol. Aliquots were then diluted 24-fold with the reaction mixture of the ATPase assay. The maximum final concentration of ethanol was 20 mM, a level which has no effect on ATPase activity under these conditions. Procedure B: Plasma membranes (8–10 mg/ml) were incubated at 25° in a 40% sucrose solution containing 0·18 M EGTA and 9 mM Tris–HCl in the presence of 0·5 M ethanol, 0·8 mM acetaldehyde or buffer. After 1 hr of incubation in a volume of 2 ml, the mixture was diluted with 38 ml of ice-cold 5 mM Tris–HCl (pH 7·5) and centrifuged at 1600 *g* for 30 min. The pellet was suspended in 40 ml of 5 mM Tris–HCl (pH 7·5) and centrifuged once more. The final pellet was suspended in an equal volume of 40% sucrose, 0·2 mM EGTA and 10 mM Tris–HCl at pH 7·5. Recovery of protein after this procedure was approximately 70 per cent, and was not affected by pretreatment with ethanol or acetaldehyde.

Disodium ATP, obtained from Sigma Chemical Co. (St. Louis, Mo.), was freed of metal ions and neutralized with Tris and $MgCl_2$ by previously described procedures [17]. EGTA and EDTA were obtained from LaMont Laboratories (Dallas, Tex.) and Mallinckrodt Chemical Works respectively. The 10^{-8} M Ca^{2+} buffer contained 25 μM $CaCl_2$ and 80 μM EGTA. Ethanol, obtained as 100% ethanol from Publicker Industries Co., was purified by redistillation. A constant boiling mixture at 79° was collected after discarding the first and last 30 per cent of the distillate. The content of ethanol was measured by the method of Bonnichsen [18]. Acetaldehyde was purchased from Eastman Kodak Co. All other reagents were of analytical grade. The concentrations of Na^+ and K^+ in the assay media were determined by flame photometry, and chlorides by chloridometry.

RESULTS

The ATPase activity of myocardial plasma membranes in the presence of 20 mM KCl and 100 mM NaCl was conspicuously inhibited by the addition of 1 M ethanol, but not 0·1 M. The higher concentration of ethanol had little effect on ATPase activity in either 120 mM NaCl or 20 mM KCl + 100 mM NaCl in the presence of 10^{-5} M ouabain (Fig. 1). Thus, ($Na^+ + K^+$)-activated ATPase was more sensitive to ethanol than was basal ATPase. Inhibition of ($Na^+ + K^+$)-activated ATPase varied with the concentration of ethanol (Fig. 2). Among four different plasma membrane preparations, 50 per cent inhibition of ($Na^+ + K^+$)-activated ATPase activity was seen at ethanol concentrations between 0·51 and 0·86 M (Table 1).

In view of the report that ethanol-induced inhibition of ($Na^+ + K^+$)-activated ATPase in the brain is competitively antagonized by K^+ [10], the effect of lowering K^+ concentration in the present cardiac preparation was examined. Inhibition of ($Na^+ + K^+$)-activated ATPase activity in 5 mM KCl + 115 mM NaCl was half-maximal at a lower concentration of ethanol (0·15 M), compared to 0·7 M ethanol for ATPase in 20 mM KCl + 100 mM NaCl. Furthermore, with ethanol

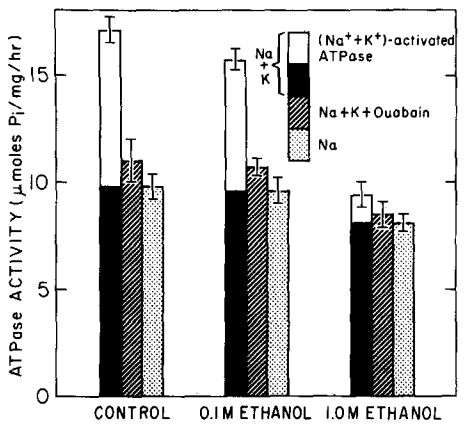

Fig. 1. Effect of ethanol on ($Na^+ + K^+$)-activated ATPase of myocardial plasma membranes. Plasma membranes of guinea-pig heart (0·2 mg/ml) were incubated in the absence and presence of 0·1 and 1·0 M ethanol. ATPase assay was performed as described in the text. Conditions: $Na^+ + K^+$, 100 mM NaCl + 20 mM KCl; $Na^+ + K^+$ + ouabain, 100 mM NaCl + 20 mM KCl + 10^{-5} M ouabain; Na^+, 120 mM NaCl. The solid portion of each lefthand bar represents the ATPase activity in 120 mM NaCl alone; the total height of the bar represents ATPase activity in 120 mM NaCl + 20 mM KCl. Value indicates ±standard deviation of nine incubations.

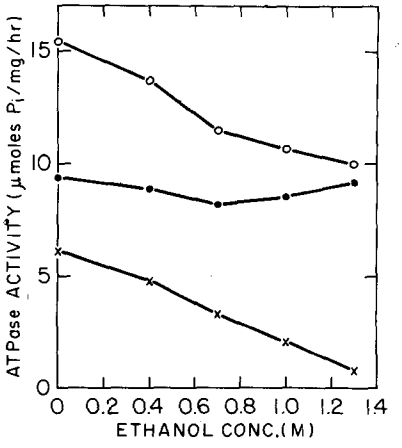

Fig. 2. Dependence of ($Na^+ + K^+$)-activated ATPase of myocardial plasma membranes on ethanol concentration. Plasma membranes (0·2 mg/ml) were incubated in standard ATPase assay media containing 100 mM NaCl + 20 mM KCl or 120 mM NaCl in the presence of various concentrations of ethanol. ○, ATPase in 100 mM NaCl + 20 mM KCl; ●, ATPase in 120 mM NaCl; ×, ($Na^+ + K^+$)-activated ATPase activity.

Table 1. Concentration of ethanol which inhibited ($Na^+ + K^+$)-activated ATPase activity by 50 per cent in four different preparations of guinea-pig myocardial plasma membranes*

Preparation No.	Ethanol concn to attain 50% inhibition (M)
1	0·64
2	0·51
3	0·76
4	0·86

* Concentrations of ethanol in the reaction mixture estimated to produce 50 per cent inhibition of ($Na^+ + K^+$)-activated ATPase activity. The plasma membranes (0·2 mg/ml) were incubated with 100 mM NaCl + 20 mM KCl or 120 mM NaCl under standard conditions described in the text.

concentrations below 0·1 M, inhibition of ATPase activity in 5 mM KCl + 115 mM NaCl was more pronounced than that in 20 mM KCl + 100 mM NaCl (Fig. 3). A significant inhibitory effect was seen at a concentration of ethanol as low as 0·1 M, in the presence of 5 mM K^+ and 115 mM Na^+.

Acetaldehyde strongly inhibited ($Na^+ + K^+$)-activated ATPase activity of plasma membranes in 5 mM KCl + 115 mM NaCl and, to a lesser extent, in 120 mM NaCl alone. The activity was inhibited progressively with increasing concentrations of acetaldehyde up to 0·8 mM, after which little further inhibition was noted up to 8 mM (Fig. 4).

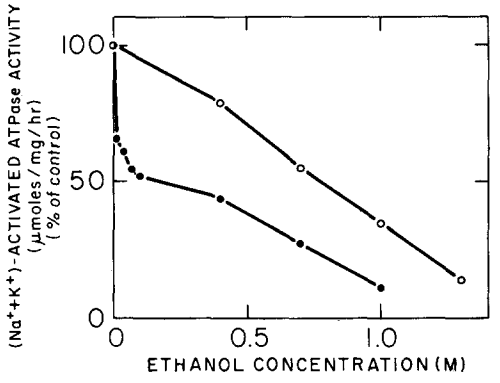

Fig. 3. Effect of KCl on ethanol-induced inhibition of ($Na^+ + K^+$)-activated ATPase activity of myocardial plasma membranes. Plasma membranes (0·2 mg/ml) were incubated in standard ATPase assay media containing 100 mM NaCl + 20 mM KCl or 120 mM NaCl in the presence of various concentrations of ethanol. ($Na^+ + K^+$)-activated ATPase activity was estimated as described in the text. ○, ($Na^+ + K^+$)-activated ATPase activity in 20 mM KCl, i.e. ATPase activity in 100 mM NaCl + 20 mM KCl minus ATPase activity in 120 mM NaCl; ●, ($Na^+ + K^+$)-activated ATPase in 5 mM KCl, i.e. ATPase in 115 mM NaCl + 5 mM KCl minus ATPase in 120 mM NaCl.

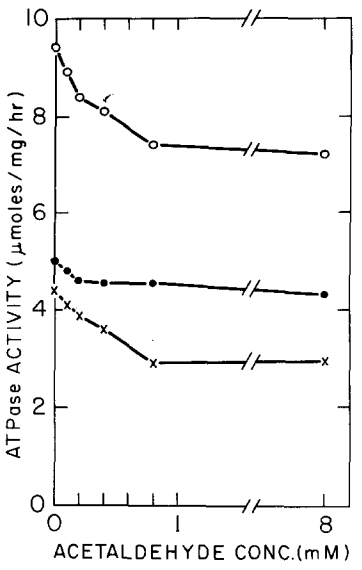

Fig. 4. Effect of acetaldehyde on the ($Na^+ + K^+$)-activated ATPase activity of myocardial plasma membranes. The plasma membranes (0·25 mg/ml) were incubated in ATPase assay media containing 115 mM NaCl + 5 mM NaCl in the presence of various concentrations of acetaldehyde. ●, ATPase activity in 115 mM NaCl + 5 mM KCl; ○, ATPase activity in 120 mM NaCl; ×, ($Na^+ + K^+$)-activated ATPase activity.

To determine if the inhibitory effect of ethanol could be accounted for by increased osmolality of the reaction mixture, the effect of adding equimolar amounts of glycerol was studied. Increasing the osmolality by the addition of glycerol had no effect on ($Na^+ + K^+$)-activated ATPase activity (Fig. 5).

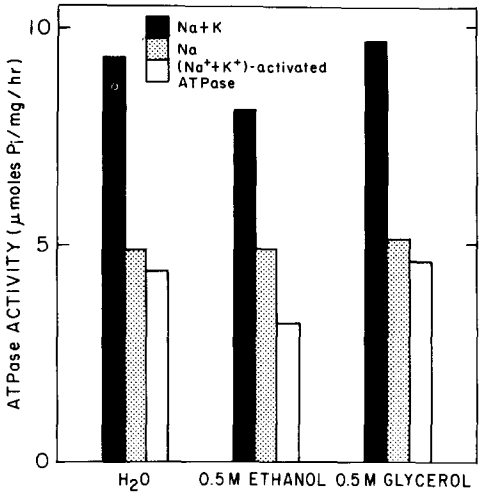

Fig. 5. A typical experiment comparing the effects of ethanol and glycerol on ($Na^+ + K^+$)-activated ATPase activity of myocardial plasma membranes. The plasma membranes (0·25 mg/ml) were incubated in ATPase assay media containing 115 mM NaCl + 5 mM KCl or 120 mM NaCl in the presence of water, 0·5 M ethanol and 0·5 M glycerol.

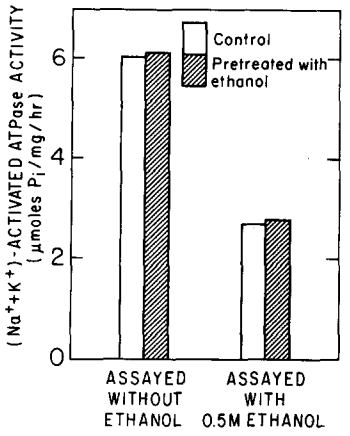

Fig. 6. Effect of ethanol on ($Na^+ + K^+$)-activated ATPase activity of myocardial plasma membranes with or without ethanol pretreatment. Pretreatment of plasma membranes was carried out as described under procedure B. ($Na^+ + K^+$)-activated ATPase activity was determined under standard conditions in the presence and absence of 0·5 M ethanol. Open bars, control plasma membranes; hatched bars, ethanol-treated plasma membranes.

To define the reversibility of the inhibitory effects, plasma membranes were incubated with or without ethanol (0·5 M) for 1·5 hr, and ATPase activity was then measured in the presence and absence of 0·5 M ethanol. The ($Na^+ + K^+$)-activated ATPase activity was not inhibited after pretreatment of plasma membranes with 0·5 M ethanol. The addition of 0·5 M ethanol to the assay medium decreased ($Na^+ + K^+$)-activated ATPase activity of both control and ethanol-pretreated membranes to a similar extent (Fig. 6). When plasma membranes were incubated with 0·5 M ethanol or 0·4 mM (Table 2) and 0·8 mM (Fig. 7) acetaldehyde, and subsequently washed with buffer according to procedure B, no differences in ($Na^+ + K^+$)-activated ATPase activity were found between control plasma membranes and those treated with ethanol or acetaldehyde.

The possibility that prolonged exposure to a low concentration of ethanol might increase the extent of

Table 2. Effects of pretreatment with ethanol and acetaldehyde on ($Na^+ + K^+$)-activated ATPase activity of myocardial plasma membranes*

Pretreatment	($Na^+ + K^+$)-activated ATPase activity (μmoles/mg/hr)
Control	4·36 ± 0·1
0·5 M Ethanol	4·21 ± 0·2
0·4 mM Acetaldehyde	4·66 ± 0·3

* The protein samples (0·25 mg/ml) were pretreated with ethanol, acetaldehyde or buffer and subsequently washed according to procedure B. ($Na^+ + K^+$)-activated ATPase activity was assayed in media containing 115 mM Na + 5 mM KCl or 120 mM NaCl.

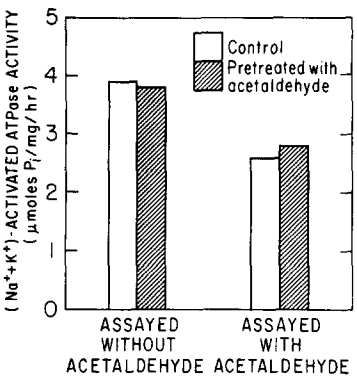

Fig. 7. Effect of pretreatment of plasma membranes with acetaldehyde. The plasma membranes were incubated with 0·8 mM acetaldehyde or buffer and subsequently washed according to procedure B. The ($Na^+ + K^+$)-activated ATPase activity of the control and acetaldehyde-treated membranes was assayed in the presence or absence of acetaldehyde (0·8 mM). The open bars represent control membranes and the cross-hatched bars represent membranes treated with acetaldehyde. The first two bars represent activity in a reaction mixture containing no acetaldehyde; the second pair of bars represents activity in the presence of 0·8 mM acetaldehyde.

inhibition of ($Na^+ + K^+$)-activated ATPase was tested by incubation of plasma membranes with 0·15 M ethanol at 25° for up to 24 hr, according to procedure A, after which ATPase activity was measured in 0·006 M ethanol. No effect of prolonged incubation was detected (Fig. 8).

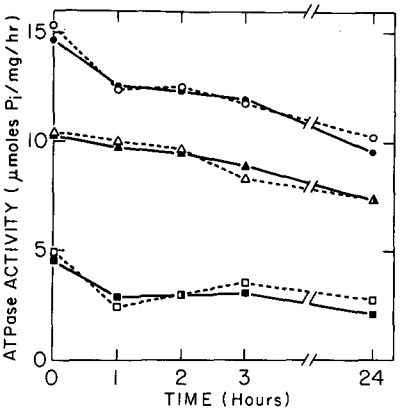

Fig. 8. Effect of ethanol pretreatment on ($Na^+ + K^+$)-activated ATPase activity of myocardial plasma membranes. Plasma membranes (6 mg/ml) were treated without or with 0·15 M ethanol at 25° under conditions described in the text. Aliquots (0·05 ml) were withdrawn at various time intervals and added to ATPase assay media containing 100 mM NaCl + 20 mM KCl or 120 mM NaCl. The concentration of plasma membranes in the ATPase assay media was 0·25 mg/ml. The concentration of ethanol, which was introduced as part of the 0·05-ml aliquot, was 6 mM. ○,●, ATPase in 100 mM NaCl + 20 mM KCl; △,▲, ATPase in 120 mM NaCl; □,■, ($Na^+ + K^+$)-activated ATPase; ○,△,□, control; ●,▲,■, pretreated with ethanol.

DISCUSSION

The finding of a dose-dependent inhibitory effect of ethanol on the ($Na^+ + K^+$)-activated ATPase activity of myocardial plasma membranes is in accord with previous reports of studies on erythrocytes, brain and skin [8, 21]. The present findings also indicate an antagonistic relationship between ethanol and K^+, as has been reported previously [10]. It has been suggested that the inhibitory effect of ethanol results from its insertion into the lipid structure of the membrane [12, 13]. Such a mode of action, by impairing the ability of K^+ to bind to the Na^+ transport system, might explain the apparent potentiation of the ethanol effect when Na^+ is partially substituted for K^+. The effects of ethanol shown in this study are not caused by denaturation or solubilization of the membranes, since the inhibition was reversible when ethanol was removed from the medium. Moreover, recovery of protein was not reduced when microsomes were centrifuged from ethanol-containing solutions [19].

The ($Na^+ + K^+$)-activated ATPase system has been ascribed a major role in the active transport of Na^+ and K^+ across cell membranes [20]. Inhibition of this membrane transport system may therefore interfere with the active efflux of Na^+ and the maintenance of the normal transcellular gradient for K^+. Impairment of the ($Na^+ + K^+$)-activated ATPase has been shown to cause gradual reduction of resting transmembrane potential by a lowering of intracellular K^+ concentration [13]. Such an effect has been shown in peripheral nerve fibers, cerebral cortical neurons and skeletal muscle fibers [13]. Other transport processes are affected by ethanol, e.g. transport of some amino acids, which has been linked to the active transport of Na^+ and K^+ [13]. A reduced absorption of a number of L-amino acids by rat intestinal sacs in the presence of different alcohols was shown by Chang *et al.* [22] and confirmed by Israel *et al.* [23]. In perfusion experiments with guinea-pig hearts, however, Schreiber *et al.* [24] found no difference in the incorporation of lysine-^{14}C.

Acetaldehyde, the primary metabolite of ethanol, has an effect similar to that of ethanol on the ($Na^+ + K^+$)-activated ATPase activity of this plasma membrane preparation; its inhibitory effect is also reversible. Despite the fact that the liver contains an abundance of enzymes which catalyze the oxidation of acetaldehyde, these systems are not entirely effective, and blood acetaldehyde levels increase after ethanol consumption in rats and man [25]. This similarity between the actions of acetaldehyde and ethanol has also been noted in studies with hepatic mitochondria [26].

The relevance of the inhibition of ($Na^+ + K^+$)-activated ATPase *in vitro* to the impairment of myocardial function that follows acute or chronic ethanol ingestion in man remains unclear. In the present studies, the concentrations of ethanol needed to inhibit membrane ATPase in 20 mM KCl + 100 mM NaCl are higher than can be achieved in the intact animal. But the potentiation of these effects at lower concentrations of K^+ indicates that the present findings may be relevant to some of the pathological effects of ethanol; the external surface of the plasma membrane, where the binding site of K^+ is located, is normally exposed to 4–5 mM K^+. The present data indicate that concentrations of ethanol that can occur *in vivo* can inhibit the ($Na^+ + K^+$)-activated ATPase when the enzyme is assayed in 5 mM K^+. Furthermore, acetaldehyde concentrations attainable *in vivo* inhibit ($Na^+ + K^+$)-activated ATPase *in vitro*.

It is difficult to attribute the negative inotropic effects of ethanol ingestion to an inhibition of ($Na^+ + K^+$)-activated ATPase, because cardiac glycosides, which are potent inhibitors of the Na^+ transport system, are associated with a positive inotropic action [27]. It should be recognized, however, that a direct causal relationship between inhibition of Na^+ transport and enhancement of contractility by cardiac glycosides is not accepted by all investigators [28]. The present findings suggest a relationship between inhibition of $Na^+ + K^+$ exchange and electrocardiographic abnormalities seen in alcoholic patients. Such a relationship remains speculative because the ST or T wave changes seen in such patients could also result from electrolyte abnormalities, epinephrine release or muscle damage, as well as from a reduction in transcellular Na^+ and K^+ gradients [29].

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