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EFFECTS OF ETHANOL ON Na, K, Mg-STIMULATED MICROSOMAL ATPase ACTIVITY*

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Abstract—Microsomal fractions containing Na, K, Mg-activated adenosinetriphosphatase activity were prepared from rat and guinea pig brain and from eel electroplaque tissue. This activity was increasingly inhibited by ethanol at concentrations (54 to 218 mM) which produce mild to severe intoxication *in vivo*. The inhibition was competitive with respect to K^+ in all three preparations. Increase in Na^+ concentration led to increased inhibition by ethanol. Ethanol intoxication in rats, measured by the inclined-plane test, was less severe when the ethanol was injected in KCl solution than when given in NaCl. This was not due to difference in blood ethanol level, and is taken as evidence that inhibition of active transport of K^+ plays an important role in ethanol intoxication *in vivo*.

It has been reported that ethanol, in concentrations that would produce mild to severe intoxication *in vivo*, inhibits the active transport of Na^+ in the isolated frog skin,[1] and the active transport of K^+ in human erythrocytes[2] and in slices of rat cerebral cortex and rabbit kidney cortex *in vitro*.[3] Ethanol could conceivably inhibit the active transport of Na^+ and K^+ in one or more of the following ways : (i) by impairment of the provision of energy to the transport mechanism, (ii) by interference with access of the ions to the carrier mechanism, or (iii) by inhibition of the carrier mechanism itself.

The first possibility was rejected because the effect of ethanol on the transport of Na^+ by frog skin was almost immediate, while that of metabolic inhibitors (cyanide, dinitrophenol) was relatively slow.[1] The second suggested mechanism implies a possible interaction of ethanol with factors such as Ca^{2+} and acetylcholine, which are thought to modify pore diameter on the surface of the cell membrane through which the ions must pass to reach the carrier system.[4] This has been investigated in part by a study of the effect of ethanol on synthesis and release of acetylcholine, which will form the substance of a separate communication. An investigation of the third possibility is described in the present paper.

There is evidence that the Na, K, Mg-activated ATPase located in the cell membrane is either the carrier involved in the active transport of Na^+ and K^+, or is closely related to it.[5,6] The fragments bearing this ATPase activity in a whole-tissue homogenate are precipitated in the 'microsomal' fraction by the ordinary method of

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differential centrifugation, and the activity has now been demonstrated in the microsomal fraction of crab nerve,[7] rat brain and kidney,[8] eel electroplaque,[9,10] and other tissues. Administration of ethanol *in vivo* has been reported to decrease the amount of Na^+ bound to the microsomal fraction subsequently obtained from rat kidney homogenates;[11] ethanol added *in vitro* caused a slight reduction in the Na^+-activated ATPase of rat brain.[12] In the latter work, however, conditions for the enzyme activity were not optimal since no K^+ was added, so that the results are inconclusive.

In the present study the effect of ethanol has been reported on the Na, K, Mg-activated ATPase of microsomal preparations from rat and guinea pig brain, and from eel electroplaque tissue. Ethanol has been found to inhibit the enzymatic activity and to show competitive antagonism with K^+. Finally, in order to see whether this effect of ethanol is related to its intoxicant action *in vivo*, a study was made of the degree of intoxication produced in rats by a standard dose of ethanol given with and without a large dose of potassium chloride.

METHODS

Preparation of microsomes from cerebral cortex

Albino rats or guinea pigs were decapitated and the brains removed as quickly as possible. The cerebral hemispheres of two animals were weighed and homogenized with ice-cold 0·25 M sucrose, buffered with 0·05 M Tris-HCl at a final pH of 7·5, in sufficient volume to give a 1 : 10 (w/v) homogenate. A Teflon-glass homogenizer with a clearance of 0·13–0·18 mm (Arthur Thomas Co.) was used, at a speed of 2,000 rev/min. Homogenization was begun within 6 min of the time of decapitation and was carried out in ten periods of 20 sec each, alternating with 40-sec periods of cooling in ice. The homogenate was centrifuged for 10 min at 2,500 rev/min (770 *g* maximum) in the SM head of a Servall RC-2 refrigerated centrifuge at 2°, to precipitate debris and nuclei. The supernatant was centrifuged in the same head at 8,000 rev/min (7,900 *g* maximum) for 15 min, to precipitate mitochondria. The new supernatant was then centrifuged in the same head at 16,500 rev/min (33,500 *g* maximum) for 60 min, to precipitate a heavy microsomal fraction. The pellet obtained was resuspended in 3 ml of the original sucrose–Tris-HCl medium. If not used the same day, it was stored at 2° and used the following day. Immediately before use it was diluted with a suitable volume of double-distilled water, usually 1 : 3 (v/v).

Preparation of microsomes from eel electroplaque tissue

The tissue,* which had been stored at −20°, was thawed and homogenized in cold 0·05 M Tris buffer, pH 7·5, in an amount sufficient to give a 1 : 10 (w/v) homogenate. An all-glass 'Duall' homogenizer (Kontes Glass Co.) was used at a speed of 2,000 rev/min for 25 periods of 20 sec each, alternating with 40-sec periods of cooling in ice. The homogenate was treated in a M.S.E. Sonicator at 10 kc/s for a total of 2·5 min, with 1-min cooling in ice after each 1 min of ultrasonic treatment. The treated homogenate was centrifuged at 2° in the SS-34 head of the Servall RC-2 centrifuge for 15 min at 8,700 rev/min (9,000 *g* maximum), and the supernatant obtained was further centrifuged at 105,000 *g* for 90 min in the no. 40 head of the Spinco model L ultracentrifuge. The microsomal pellet was resuspended in 0·20 M Tris-HCl buffer at

* We are greatly indebted to Dr. R. W. Albers, of the National Institute of Neurological Diseases and Blindness, Bethesda, Md., for a generous gift of frozen electroplaque tissue.

pH 7·5 and tested for ATPase activity within 3 days. Approximately 35% of the total Na, K, Mg-stimulated ATPase activity of the initial homogenate was recovered in the final microsomal suspension. When stored at 0° the preparation lost 15 to 20% of its activity per day.

Adenosine triphosphatase assay

The complete reaction mixture contained 70 mM Tris-HCl buffer at pH 7·5, 5 mM Tris–ATP (Sigma), 5 mM $MgCl_2$, and NaCl and KCl in concentrations that varied in different experiments. The mixture was preincubated for 5 to 10 min before addition of enzyme. The final volume in each tube after addition of the enzyme preparations was 1·0 ml. Incubations were carried out at 30° with eel tissue microsomes, and at 37° with rat and guinea pig brain microsomes. The tubes were covered with Parafilm to decrease evaporation of ethanol. After 20 min the reaction was stopped by addition of 2·0 ml of cold 5% trichloracetic acid, and the precipitated protein was separated by centrifugation. Inorganic phosphate was measured in 2·0 ml of supernatant by the method of Fiske and SubbaRow.[13] Enzyme activity was expressed as μmoles P_i released by the microsomes obtained from 1 g original tissue in 1 hr (μmoles P_i/g/hr). For each preparation, control values were obtained by stimulating the enzyme with Mg^{2+} in the absence of K^+ and Na^+ (MgATPase), and with Mg^{2+}, K^+, and Na^+ together. NaKMg-ATPase activity was obtained by difference between these values.

Measurement of intoxication by the sliding-angle technique

The degree of intoxication produced in rats by the administration of ethanol was measured by the inclined-plane method.[14] To decrease the variability of the results, the free end of the tilting board was raised at a constant angular velocity by means of an electric motor and pulleys. The angle at which the rat began to slide was measured three times in quick succession, and the values were averaged to give a pretreatment base line. Each rat then received an i.p. injection of a 20% (w/v) solution of redistilled ethanol in a dose of 1 ml/100 g body weight, i.e. 2 g absolute ethanol/kg. For the control tests the ethanol solution contained 0·5 M NaCl; for the experimental runs it contained 0·5 M KCl. Thirty min after the injection, the sliding angle was again measured three times in succession and averaged. When intoxicated, the rat slides at a lower angle than it does normally. The degree of intoxication is conveniently expressed as the per cent decrease from pretreatment to posttreatment sliding angle.

Determination of ethanol and potassium in blood

Subsequent to the intoxication experiments *in vivo*, two groups of 12 rats each received either ethanol in NaCl solution or ethanol in KCl solution, by intraperitoneal injection, in dosage corresponding exactly to that used in the tilting-plane tests. At 30 min after injection, corresponding to the time of the sliding-angle measurements, 0·1-ml samples of blood were obtained from the tails. The samples were deproteinized with $ZnSO_4$–NaOH[15] and analysed for ethanol by the Smith and Newman modification of the alcohol dehydrogenase method.[16] A standard curve with known concentrations of pure ethanol was made each day, and the ethanol concentrations of the blood samples were obtained by interpolation on the graph.

In a separate experiment, 12 rats received i.p. injections of 1·0 ml 0·5 M KCl/100 g body weight, and 12 others received an equal dose of 0·5 M NaCl solution. After 30 min the rats were decapitated, and individual blood samples were caught in heparinized tubes. After centrifugation, the plasma samples were analysed for K^+ in a Baird DB-2 flame photometer, by the Li^+ internal standard method.

RESULTS

ATPase activity of brain and electroplaque preparations

The characteristics of the microsomal ATPase activities were as described in the literature.[7,9,10,17] A typical preparation from either rat or guinea pig brain had a NaKMg–ATPase activity ranging from 30 to 50 μmoles P_i/g/hr. Under optimum conditions of Na^+ and K^+ concentration, the ratio of NaKMg–ATPase to Mg–ATPase varied from 1·5 to 2·0. The NaKMg–ATPase activity of a typical electroplaque preparation ranged from 75 to 150 μmoles P_i/g/hr, and the ratio of NaKMg–ATPase to Mg-ATPase varied between 15 and 20.

The effect of ouabain was tested on two preparations. Addition of 5×10^{-4}M ouabain to a crude homogenate of eel electroplaque tissue reduced the NaKMg-stimulated ATPase activity by 76%. With a preparation of microsomes from electroplaque tissue, in a medium containing 10 mM K^+ and 100 mM Na^+, 5×10^{-4}M ouabain produced 82% inhibition of the NaKMg–ATPase activity.

Both Na^+ and K^+ were required for maximal activity of the microsomal preparations. Variation of the Na/K ratio between 29 and 0·15, at a total $Na^+ + K^+$ concentration of 150 mM, did not affect the activity greatly, but marked reduction occurred when one or the other cation was removed completely.

Effects of ethanol on ATPase activity

The effect of ethanol upon the ATPase activity of the microsomal preparations was first studied at fixed concentrations of Na^+ and K^+, with ethanol concentrations ranging from 0·25 to 1·0% (54 to 218 mM). As shown in Fig. 1, ethanol inhibited the NaKMg–ATPase activity of all three preparations, the degree of inhibition

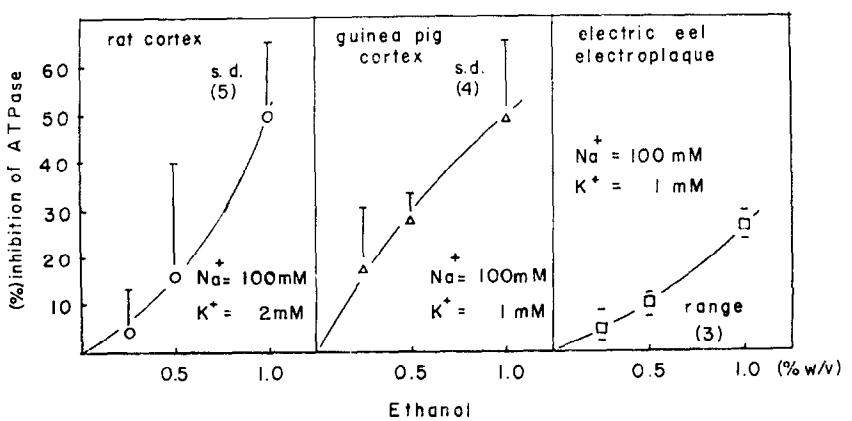

FIG. 1. Effects of different concentrations of ethanol on NaKMg-stimulated ATPase activity of microsomal preparations from rat cerebral cortex, guinea pig cerebral cortex, eel electroplaque.

increasing with the concentration of ethanol. The concentrations of ethanol indicated in Fig. 1 for electric eel electroplaque are only approximate because, after completion of this series of experiments, it was discovered that the ethanol stock solution contained only about 50% of the intended concentration, and the experiments could not be repeated with a new solution because all the eel tissue had been used. However, the relative concentrations of ethanol shown in the graph are valid. The inhibitory effect of ethanol was relatively specific for the NaKMg–ATPase. Thus the inhibition produced by 218 mM ethanol with the rat brain preparation (Fig. 1) was 50 ± 13% (S.D.) for the NaKMg–ATPase but ranged from 0 to 8% for the ouabain-insensitive Mg–ATPase.

In order to clarify the nature of the inhibition produced by ethanol, samples of a preparation of eel electroplaque microsomes were exposed to 0·22 M ethanol, in a medium containing 100 mM Na^+ and 1 mM K^+, for different lengths of time before addition of ATP, and the activities of NaKMg-activated ATPase were expressed as percentages of the control value for the same preparation without ethanol. The degrees of inhibition found after different lengths of pre-exposure to ethanol were 24%, 30%, and 20% after 15 min, 7·5 min, and 0 min respectively. As can be seen, pre-exposure to ethanol did not enhance the degree of inhibition. It is most unlikely, therefore, that the inhibition depends on denaturation of the enzyme by ethanol.

The ethanol effect was then studied at fixed concentrations of Na^+ (100 mM) and of ethanol (218 mM), with potassium concentrations varying from 1 to 10 mM for the brain preparations and from 0·5 to 20 mM for the electroplaque preparations. In all three systems there was evidence of competitive antagonism between ethanol and K^+, as shown by Lineweaver-Burk reciprocal plots of 1/activity vs. $1/K^+$ (Figs. 2–4). With the rat brain NaKMg–ATPase, the K^+ concentration required for half-maximal activity rose from $2{\cdot}2 \times 10^{-3}$M in the absence of ethanol to $5{\cdot}0 \times 10^{-3}$M in the presence of 218 mM ethanol (Fig. 2). The corresponding rise in K_m for the guinea pig brain enzyme was from $2{\cdot}1 \times 10^{-3}$ to $3{\cdot}7 \times 10^{-3}$M (Fig. 3). With the eel

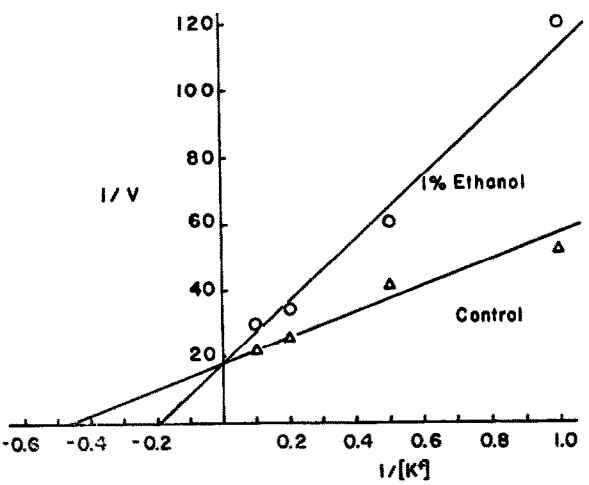

FIG. 2. Lineweaver-Burk plot of reciprocals of NaKMg-stimulated ATPase activity (μmoles P_i/g tissue/hr $\times 10^3$) and K^+ concentration (mM) for rat cerebral cortex microsomes. Each point represents the average of five different preparations. Na^+ concentration was 100 mM, and ethanol was 0·22 M.

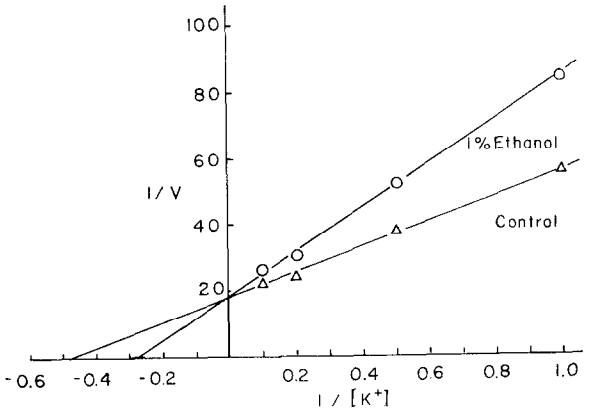

FIG. 3. Lineweaver-Burk plot of NaKMg–ATPase activity and K^+ concentration for guinea pig cerebral cortex microsomes. Units and conditions as in Fig. 2. Each point represents the average of two preparations.

electroplaque preparation, the K_m rose from $7{\cdot}0 \times 10^{-4}$M K^+ to $1{\cdot}25 \times 10^{-3}$M on addition of 109 mM ethanol (Fig. 4).

In another set of experiments, the effect of 1% ethanol (0·22 M) on eel electroplaque microsomes was studied at fixed K^+ concentrations and varied Na^+ concentrations. Figure 5 shows the results with 1 mM K^+ when Na^+ varied between 5 and 160 mM. As can be seen, maximal activation of the NaKMg–ATPase activity occurred at a Na^+ concentration of 20 mM, and higher Na^+ concentrations produced some inhibition. There was, however, a steady increase in both the relative and absolute inhibitory effect of ethanol with rising Na^+ concentration. When K^+ was held constant at 40 mM, however, there was no observable effect of ethanol at any Na^+ concentration between 5 and 240 mM (Fig. 6).

Effect of potassium on ethanol intoxication in vivo

Thirty-six adult female Wistar rats, weighing 200–250 g each, were divided into six equal groups. Each rat was tested on two occasions, a minimum of 3 days apart. On one occasion the impairment in sliding angle was determined after administration

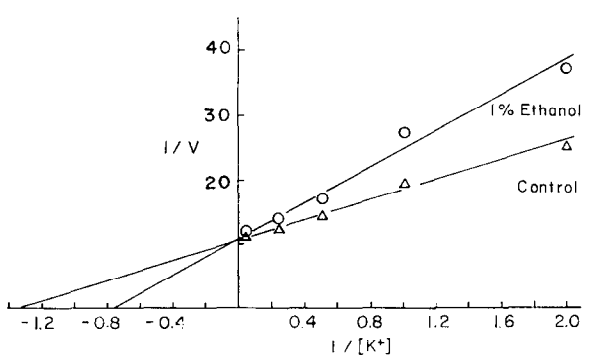

FIG. 4. Lineweaver-Burk plot of NaKMg–ATPase activity and K^+ concentration for eel electroplaque microsomes. Each point represents the average of five preparations. Units and conditions as in Fig. 2.

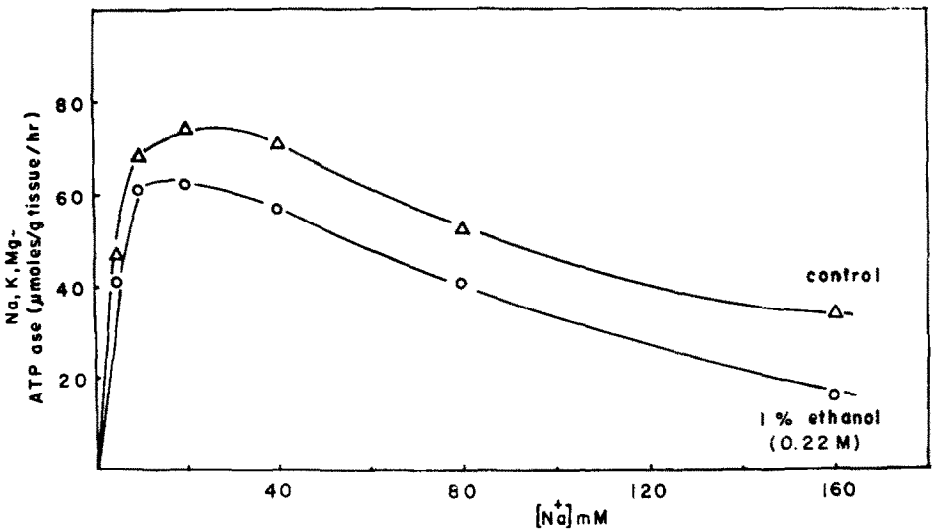

FIG. 5. Effect of 0·22 M ethanol on NaKMG-stimulated ATPase activity of eel electroplaque microsomes at different Na^+ concentrations. K^+ concentration was 1 mM in all cases.

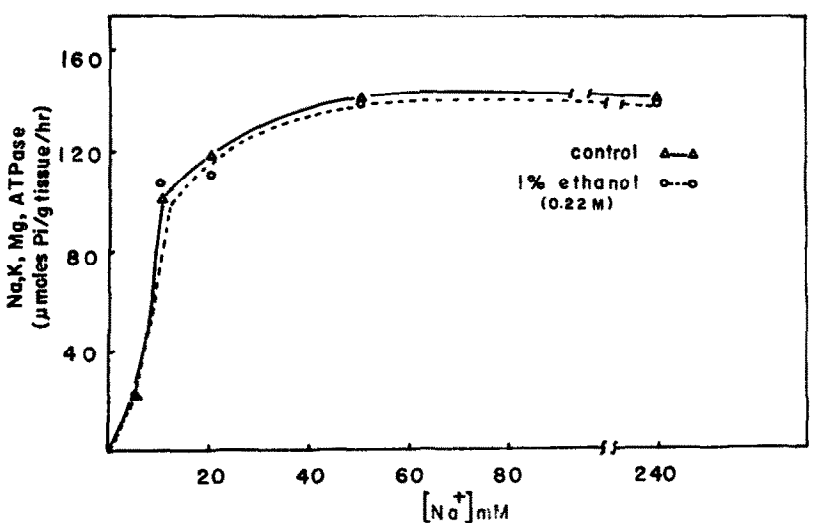

FIG. 6. Effect of 0·22 M ethanol on NaKMg-stimulated ATPase activity of eel electroplaque microsomes at different Na^+ concentrations, and fixed K^+ concentration of 40 mM.

of ethanol, 2 g/kg in 0·5 M NaCl as described in Methods, and on the other occasion after the same dose of ethanol in 0·5 M KCl. For groups 1, 3, and 5 the test with KCl was done first, and the test with NaCl second. For groups 2, 4, and 6 the order of the tests was reversed. Table 1 shows the values obtained with each rat, expressed as per cent impairment observed in each test. Table 2 is a summary of the analysis of variance of these results, and shows that the mean value for per cent impairment was significantly less when the ethanol was given in KCl (27% impairment) than when

given in NaCl (32% impairment). It was also noted that the rats were considerably less somnolent and more active when the KCl was employed.

As a control for the sliding-angle tests, 12 additional rats were tested on two occasions each, once before and after receiving 1·0 ml 0·5 M NaCl/100 g body weight, and once before and after an equal volume of 0·5 M KCl. The mean value and

TABLE 1. COMPARISON OF DEGREE OF INTOXICATION PRODUCED BY ETHANOL (2g/kg) INJECTED WITH NaCl AND WITH KCl*

Group	First trial				Second trial			
	Salt	Sliding angle		Impairment %	Salt	Sliding angle		Impairment (%)
		Control	Alcohol			Control	Alcohol	
1	KCl	55	50	8	NaCl	61	36	40
		60	40	33		62	34	45
		54	46	15		57	43	25
		64	47	26		64	34	47
		52	43	17		54	39	28
		59	53	10		56	37	34
			mean	18·2			mean	36·5
2	NaCl	53	37	30	KCl	47	40	15
		52	35	33		55	36	34
		50	41	18		46	38	18
		58	35	40		62	40	36
		55	31	44		51	34	33
		47	35	26		46	37	20
			mean	31·8			mean	26
3	KCl	57	45	20	NaCl	59	40	32
		57	45	20		52	34	35
		59	51	14		62	36	42
		58	37	36		55	35	37
		62	46	26		51	46	10
		59	39	33		60	40	33
			mean	24·8			mean	31·5
4	NaCl	57	41	30	KCl	55	35	36
		55	34	38		60	36	40
		63	46	27		58	36	38
		64	43	33		61	42	31
		59	40	32		59	36	39
		62	36	40		56	35	37
			mean	33·3			mean	36·8
5	KCl	53	41	23	NaCl	46	43	7
		63	42	33		58	42	28
		58	43	26		56	41	27
		55	40	27		57	37	35
		63	39	38		59	47	21
		51	37	27		54	40	26
			mean	29			mean	24
6	NaCl	63	40	37	KCl	60	41	32
		58	38	34		59	47	21
		61	38	38		60	38	37
		59	37	37		63	47	26
		56	38	32		58	39	33
		62	49	21		60	48	20
			mean	33·2			mean	28·2

* Overall averages: impairment NaCl = 32%; impairment KCl = 27%.

standard deviation for the postinjection value after NaCl was 100·7 ± 5·0% of the pretreatment value; the corresponding value for the KCl tests was 99·3 ± 3·2%; Thus, neither salt solution alone produced any change in the sliding angle.

Blood samples obtained from the tail veins of rats 30 min after the administration of ethanol in NaCl or KCl solution were analysed for ethanol concentration. The means and standard deviations for the 12 rats in the ethanol + NaCl test were 196 ± 18 mg/100 ml, and 186 ± 15 mg/100 ml for the ethanol + KCl test. These values are not significantly different.

TABLE 2. SUMMARY TABLE OF ANALYSIS OF VARIANCE OF IMPAIRMENT VALUES RECORDED IN TABLE 1

Source	D.F.	S.S.	M.S.	F ratio
Animals	35	3,245·8	93·0	
Order of trials	1	80·2	80·2	
K^+ vs. Na^+	1	373·6	373·6	5·67*
Error	34	2,242·2	65·9	

* F ratio for K^+ vs. Na^+ is significant: $P < 0·025$.

Finally, serum potassium levels in 12 rats, 30 min after injections of NaCl solution, averaged 6·60 ± 0·90 mEq/liter. In 12 rats receiving the KCl solution, the corresponding value was 9·40 ± 1·60 mEq/liter.

DISCUSSION

The relatively strong inhibitory effect of ethanol upon three different microsomal NaKMg-activated ATPases in the present study parallels its previously noted inhibitory effect upon active transport of Na^+ and K^+ in isolated tissues.[1–3] These enzyme preparations were derived from three different species and two tissues, including one of the tissues in which comparable concentrations of ethanol had earlier been shown to inhibit active transport of potassium.[3] Together with the evidence cited in the introduction concerning the central role of the microsomal NaKMg-stimulated ATPase in cation transport, these findings suggest that ethanol is probably capable of depressing the membrane ATPase activity in most or all types of animal cell.

The present findings also indicate that in all three cases and, therefore, presumably also with other such NaKMg–ATPase preparations from other tissues, ethanol exerts a concentration-dependent effect that is competitively antagonized by K^+. This antagonism appears to be relatively specific, since the effect of ethanol was not altered in the same way by changes in Na^+ concentration. An increase in Na^+ concentration either increased the inhibitory effect of ethanol (Fig. 5) at low K^+ levels or had no effect at high K^+ level (Fig. 6). In no case did an increase in Na^+ concentration decrease the inhibitory effect of ethanol.

The mechanism of competition between ethanol and K^+ is not clear, since it seems rather improbable that they would be competing for the same receptor site, in view of their different particle size and the absence of a charge on the ethanol molecule. Because of the well-known relation between lipid-solubility (or the related

value for thermodynamic activity in the lipid phase) and intoxicating potency in the homologous series of primary aliphatic alcohols,[18–20] it might be reasonable to conjecture that ethanol occupies a lipophilic site on a component of the ATPase system, while K^+ occupies a hydrophilic site on the same protein. If ethanol, in occupying its site, causes reversible alteration in configuration such as to give rise to an induced misfit at the K^+ site, and K^+ produces a reciprocal misfit at the ethanol site, this would satisfactorily reconcile the lipid-solubility observations with the competitive antagonism between ethanol and K^+. Pauling[21] has recently pointed out that the Meyer–Overton series does not necessarily mean that the drugs act in a lipid phase of the cell membrane, because the molecular properties which determine lipid solubility also give rise to the tendency to form clathrate compounds involving the aqueous medium and polar groups on the cell membrane. Evidence has been presented[22] in support of this mechanism in the action of a number of gaseous anaesthetics. Our conjectured explanation of the competitive antagonism between ethanol and K^+ is quite compatible with this hypothesis, if it is assumed that clathrate formation involving ethanol gives rise to the suggested misfit at the K^+ site.

Some support for this concept may be found in the observation that urea produces a reversible competitive inhibition of xanthine oxidase.[23] While this could conceivably reflect a direct competition for a common binding site, the quantitative relationships are such as to suggest that the action of urea is more likely exerted through a modification of the tertiary structure of the enzyme, leading to distortion of the xanthine site.

The inhibition of NaKMg–ATPase of guinea pig brain microsomes produced by 108 mM ethanol in the presence of 1 mM K^+ is of the same order as the previously noted inhibition of K^+ uptake by guinea pig cortex slices in a medium containing 6 mM K^+.[3] On the other hand, Whittam has shown[24] that activation of the NaKMg–ATPase of erythrocyte membranes is a function of extracellular rather than intracellular K^+ and, *in vivo*, the extracellular K^+ concentration is approximately 6 mM. These facts are not necessarily in conflict. The ATPase preparation consists of fragments that offer little or no diffusion barrier, whereas brain cortex slices (especially in the experiments involving net gain of K^+ from the medium) may well offer such a barrier that could limit access of K^+ to the active transport site. It is quite conceivable that at an eternal K^+ concentration of 6 mM, the concentration at the membrane ATPase site is 2 mM or less. This point can not be settled until direct measurement of the latter is possible.

If the observed inhibition of the NaKMg–ATPase by ethanol does occur *in vivo*, and results in inhibition of active transport of Na^+ and K^+, it remains to be shown that this action contributes to the characteristic intoxicant effects of ethanol. The role of cation movements in the conduction of the nerve impulse[25] and their suggested role in the generation of both excitatory and inhibitory postsynaptic potentials[26] are well known, and it is a reasonable conjecture that impairment of cation transport by ethanol would ultimately impair both axonal conduction and synaptic transmission. There is abundant evidence that ethanol blocks the increased respiration by brain cortex slices resulting from increased active transport of Na^+ and K^+ following electrical[27] or chemical[28, 29] stimulation.

The most direct evidence to date, however, is provided by the finding that a deliberate elevation of the extracellular K^+ concentration decreased the degree of intoxication produced by a constant dose of ethanol (Table 2). The dose of KCl

used for this purpose had no demonstrable effect by itself on the performance of rats which received no ethanol. While the mean improvement in performance associated with the administration of KCl was small (27% impairment with ethanol + KCl vs. 32% impairment with ethanol + NaCl), it was statistically significant ($P < 0{\cdot}025$). It should be noted that the increase in serum K^+ level was small, the dose having been selected to avoid the risk of cardiac arrest due to hyperpotassemia. It should also be noted that this dose of potassium, by reducing the ethanol effect from 32% impairment to 27% impairment, was actually decreasing the ethanol effect by 16% {i.e. [(32 − 27)/32) × 100%}. The potassium did not affect the blood level of ethanol produced by the standard dose, nor did it modify performance in the sliding test in the absence of ethanol, so it seems reasonable to assume that it antagonized the action of ethanol directly on the nervous system.

If the intoxicant effect of ethanol is directly related to its action on cation transport, it might be expected that ouabain and other drugs that inhibit NaKMg–ATPases would cause similar intoxicant effects. Ouabain does not do so acutely *in vivo*, though confusion, drowsiness, and other central nervous system disturbances can occur as chronic toxic effects. Perhaps the lack of acute nervous effects is due to the fact that cardiac toxicity may prove lethal too early. Wallgren has reported that ouabain *in vitro*, shows neither potentiation nor addition of effects with ethanol in the suppression of electrically stimulated respiration of brain cortex slices.[30] This is not necessarily in conflict with our findings concerning ethanol and NaKMg–ATPases, which are known to be inhibited by ouabain also. Although the stimulation of Na^+ and K^+ transport is followed by a stimulation of respiration, the chain of connection between ion transport and respiration includes several links. As Wallgren suggests, ethanol and ouabain may both inhibit ion transport, yet affect respiration differently.

Of more interest is the finding that chlorpromazine and the antihistaminics, promethazine and diphenhydramine, inhibit Na^+ and K^+ transport in liver slices, inhibit the NaKMg–ATPases in liver and brain microsomes, and show competitive antagonism with respect to K^+.[31] These effects are exactly like those of ethanol on the brain preparations, and antihistaminics and chlorpromazine are known to enhance the depressant effect of ethanol *in vivo*.[32, 33] It would be of interest to carry out similar studies on brain preparations with other drugs, such as meprobamate and other tranquilizers, which enhance the effect of ethanol *in vivo*.[32]

Since ethanol appears to inhibit Na^+ and K^+ transport in many tissues, its apparently selective action upon the nervous system, and even upon certain parts of the nervous system, requires explanation. It is not likely due to selective distribution, since ethanol is fairly rapidly distributed throughout all body water,[34] and ^{14}C-ethanol has been shown to distribute uniformly throughout the brain water.[35] Therefore one may conjecture that the NaKMg–ATPases of different tissues differ in their affinity for ethanol, or that local concentrations of K^+ vary in the microenvironments of these ATPases, or that the tissues differ with respect to the functional importance of the NaKMg–ATPase which they contain. There is not yet sufficient evidence to permit a final decision concerning this question.

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