Biochemical Pharmacology, Vol. 35, No. 12, pp. 1987–1992, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

EFFECTS OF SHORT-CHAIN ALCOHOLS AND NOREPINEPHRINE ON BRAIN (Na^+,K^+)ATPase ACTIVITY

PATSON T. NHAMBURO,*†‡ BERNARD P. SALAFSKY,* PAULA L. HOFFMAN† and BORIS TABAKOFF†

*University of Illinois College of Medicine at Rockford, Rockford, IL 61107; and †Laboratory for Studies of Neuroadaptive Processes, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD 20205, U.S.A.

(*Received* 2 *July* 1985; *accepted* 3 *October* 1985)

Abstract—(Na^+,K^+)ATPase activity in synaptic membranes from whole brains of mice was inhibited by a series of short-chain aliphatic alcohols (ethanol through pentanol). The relationship of inhibitory potency to alcohol chain length and to alcohol membrane:water partition coefficient suggested that the inhibitory effect of the alcohols does not depend totally on their interaction with neuronal membrane lipids. Although partitioning into the membranes is important for this inhibitory effect, a direct interaction of alcohol with the enzyme protein may also be involved in the inhibition. Norepinephrine did not significantly potentiate inhibition of (Na^+,K^+)ATPase activity by low concentrations of ethanol in preparations of either mouse or rat brain. Thus, under our conditions, ethanol, at levels which can be reached *in vivo*, only slightly inhibited enzyme activity, and the possible importance of this inhibition in mediating the *in vivo* acute or chronic effects of ethanol on the CNS remains open to question.

Ethanol inhibits the activity of sodium-potassium-activated adenosine triphosphatase [(Na^+,K^+)-ATPase] in membrane preparations from mouse [1, 2] and rat [3, 4] brain. (Na^+,K^+)ATPase is a membrane-bound enzyme whose activity can also be altered by changes in its lipid microenvironment [5, 6]. Since ethanol increases the "fluidity" of brain membranes [7], it has been postulated that the inhibitory effect of ethanol on (Na^+,K^+)ATPase activity occurs secondarily to ethanol-induced changes in the properties of the lipids surrounding the enzyme [1, 8]. This hypothesis is supported by the findings that high concentrations of ethanol are necessary both to inhibit enzyme activity and to increase membrane fluidity [1, 3, 9]. However, when membrane fluidity is increased by other means, such as increased temperature or changes in phospholipid:cholesterol ratio, (Na^+,K^+)ATPase activity is *increased* [10, 11]. It has, therefore, also been suggested that inhibition of (Na^+,K^+)ATPase activity by ethanol may result from direct interactions of ethanol with the enzyme protein [4] or from solvent effects [12], rather than from effects of ethanol on the properties of membrane lipids. In the present study, we have examined the effects of several short-chain aliphatic alcohols on (Na^+,K^+)ATPase activity in mouse synaptic membranes, in order to further elucidate the mechanism by which ethanol inhibits enzyme activity.

We have also investigated another aspect of ethanol-induced inhibition of (Na^+,K^+)ATPase activity, i.e. sensitization to ethanol-induced inhibition by catecholamines [4, 13, 14]. In rat brain membranes, norepinephrine (NE) enhanced the inhibition of (Na^+,K^+)ATPase caused by ethanol, reportedly by facilitating an ethanol-induced conformational change [13]. In the presence of NE, physiologically-attainable ethanol concentrations produced substantial inhibition of (Na^+,K^+)ATPase activity, suggesting that, *in vivo*, ethanol could specifically inhibit enzyme activity in brain areas which contain noradrenergic innervation [13, 14]. We wished to determine whether this interaction occurs in mouse brain as well as in rat brain, and may therefore be generally considered as a possible mechanism mediating the CNS effects of ethanol.

‡ Send correspondence to: Patson T. Nhamburo, Ph.D., Laboratory for Studies of Neuroadaptive Processes, NIAAA, 12501 Washington Ave., Rockville, MD 20852.

MATERIALS AND METHODS

Tissue preparation. Male C57Bl mice (22–25 g) and male Wistar rats (250–300 g) were used in these experiments. Animals were group-housed in our laboratories under conditions of constant temperature and lighting (12-hr light/dark cycle) for at least 7 days prior to beginning experiments. Mice were killed by decapitation, and brains were dissected in the cold. A synaptosomal preparation of whole brain (minus cerebellum and spinal cord) was obtained by the method of Cotman [15], using sucrose–Ficoll density gradient centrifugation. Synaptosomal pellets were resuspended in 3-[*N*-morpholino]propanesulfonic acid buffer (MOPS–NH_4^+) (50 mM, pH 7.4) and frozen overnight. Prior to assays of (Na^+,K^+)-ATPase activity, the suspension was thawed and subjected to hypoosmotic shock (20 min in distilled water at 0°) and then centrifuged at 17,000 *g* for 20 min. The synaptic membrane pellet was resuspended in 50 mM MOPS–NH_4^+ (pH 7.4) to give a final protein concentration of about 1.0 mg/ml [16].

For studies of NE effects, cerebral cortical tissue from mice or rats was homogenized in 0.25 M sucrose containing 20 mM Tris–HCl, 1 mM Na_2EDTA and 0.1% deoxycholate, pH 7.4, and a crude synap-

tosomal membrane pellet was prepared [15]. This pellet was washed twice in the same buffer without detergent, and then diluted 10-fold with cold distilled water, and assayed for (Na^+,K^+)ATPase activity. This membrane preparation was identical to that used in prior studies of the effects of catecholamines [4, 13, 14].

(Na^+,K^+)ATPase assay. (Na^+,K^+)ATPase activity was determined as detailed previously [1, 17]. Two sets of reactions were assayed simultaneously. In one set, the assay mixture contained, in a total volume of 1.0 ml, MOPS–NH_4^+ (50 mM, pH 7.4), $MgCl_2$ (5 mM), NaCl (100 mM), KCl (5 mM), Tris–ATP (5 mM; Sigma Chemical Co., vanadium-free) and 50–100 μg of synaptic membrane protein. An otherwise identical assay mixture, but without NaCl and KCl, was used to estimate the Mg^+-ATPase activity. Blanks contained all components except for tissue. Reaction mixtures were prepared at 0° and preincubated for 10 min at the appropriate temperature (10–37° for Arrhenius plots, or 37° when ethanol inhibition was studied) prior to starting the reaction. The reaction was initiated by the addition of Tris–ATP, and incubations were continued for 30 min (10–15°), 20 min (16–25°) or 10 min (25–37°). Ice-cold 0.05 M silicotungstic acid (0.5 ml) was added to terminate the reaction. Inorganic phosphate was extracted and quantitated as described by Penniall [18]. (Na^+,K^+)ATPase activity was calculated as the difference between the quantity of inorganic phosphate liberated in the presence and in the absence of Na^+ and K^+.

In studies of the effect of NE on the enzymic activity, incubation conditions were modified in order to be identical to those used previously [4, 13, 14]. The assay mixture contained, in a total volume of 1.3 ml, imidazole (30 mM), glycylglycine (30 mM), $MgCl_2$ (3 mM), Tris–ATP (3 mM; vanadium-free), NaCl (120 mM), KCl (5 mM) and 0.01 ml (about 100 μg protein) of diluted crude synaptosomal membranes (pH 7.4). In another set of reaction mixtures, ouabain (1 mM) was added and the monovalent cations were omitted. The reaction was started by addition of the enzyme, and incubations were carried out for 20 min at 37° in a shaking water bath. Cold 0.05 M silicotungstic acid (0.5 ml) was added to terminate the reaction, and samples were centrifuged at 2000 *g* for 10 min. The quantity of inorganic phosphate in the supernatant fraction was then estimated, and (Na^+,K^+)ATPase activity was calculated as the difference between the quantity of inorganic phosphate liberated in the presence and in the absence of ouabain.

K^+-dependent p-nitrophenylphosphatase ("phosphatase") assay. This enzyme activity was measured according to the method of Swann *et al.* [19]. Incubations, in a total volume of 0.5 ml, contained Tris–HCl (50 mM, pH 7.5), $MgCl_2$ (5 mM), *p*-nitrophenylphosphate (10 mM; Tris salt), 100 μg of synaptic membrane protein, and various concentrations of KCl, ethanol and NE as described in Results. The reaction mixtures were prepared at 0° and preincubated for 10 min at 30°; the reaction was initiated by addition of *p*-nitrophenylphosphate. After a 10-min incubation at 30°, the reaction was terminated with 2 ml of ice-cold 0.1 M NaOH, and *p*-nitrophenol was determined spectrophotometrically by measurement of absorption at 410 nm. Activity without K^+, or in the presence of 5 mM ouabain, was subtracted from that with K^+ to obtain K^+-dependent activity. In some experiments, LiCl (30 mM) replaced KCl in order to assay Li^+-dependent phosphatase activity, which has been reported to be a direct measure of the catalytic activity of (Na^+,K^+)ATPase [10].

Addition of alcohols and NE in assays of (Na^+,K^+)ATPase and phosphatase activities. Alcohols were added to the reaction mixture at the beginning of the preincubation period, and tubes were capped throughout the assay. Norepinephrine hydrochloride [(−)-Arterenol; Sigma] was dissolved in either ascorbic acid (10^{-8} M) or acidic distilled water (pH 4.0), and only freshly prepared solutions were used.

Statistical analysis. Arrhenius parameters were determined by computer analysis as previously described [1], and statistical comparisons were made by Student's *t*-test. $P < 0.05$ was chosen as the level of significance.

RESULTS

Inhibition of (Na^+,K^+)ATPase by alcohols in vitro. All of the alcohols tested (1-pentanol, 1-butanol, 1-propanol and ethanol) inhibited (Na^+,K^+)ATPase activity in a dose-dependent manner. The sensitivity of (Na^+,K^+)ATPase activity to each alcohol can be represented by the slope of each dose–response curve. These values were: ethanol, 0.05; 1-propanol, 0.20; 1-butanol, 0.43; and 1-pentanol, 1.20. Thus, the efficacy of alcohols in inhibiting enzyme activity varied over about a 20-fold range from ethanol to 1-pentanol, and the inhibitory potency of alcohols increased with increasing chain length. The relationship between sensitivity of the enzyme to inhibition and alcohol chain length is shown in Fig. 1A. The potency of the alcohols increased logarithmically with the number of methylene groups, and the slope of the regression line was 0.45 ($r = 0.99$). A similar plot for Mg^{2+}-ATPase had a slope of 0.18 ($r = 1.0$; data not shown). The logarithm of the inhibitory potency of the alcohols was also linearly related to the logarithm of the membrane:water partition coefficients of the alcohols. In this instance, the slope of the regression line was 0.82 ($r = 0.99$; Fig. 1B).

Arrhenius plots: Effect of ethanol or 1-butanol. Plots of the temperature dependence (Arrhenius plots) of mouse brain (Na^+,K^+)ATPase activity were characterized by discontinuities, i.e. an abrupt change in slope occurring at 23.8 ± 0.4° (mean ± S.D.; N = 6), as has been reported previously [1, 20] (Fig. 2). This transition temperature (T_t) was reduced significantly on addition to the assay of either 500 mM ethanol (to 21.3 ± 1.2°; $P < 0.01$) or 98 mM 1-butanol (to 19.5 ± 0.7°; $P < 0.001$) (Fig. 2). These concentrations of ethanol and butanol were calculated to result in the same intramembrane alcohol concentration (62.5 mM) [21]. Ethanol and 1-butanol also significantly reduced the activation energy below T_t (i.e. E_{a1}) by 18 and 27% respectively (Fig. 2).

Norepinephrine effects on (Na^+,K^+)ATPase activity and on ethanol inhibition of activity. In pre-

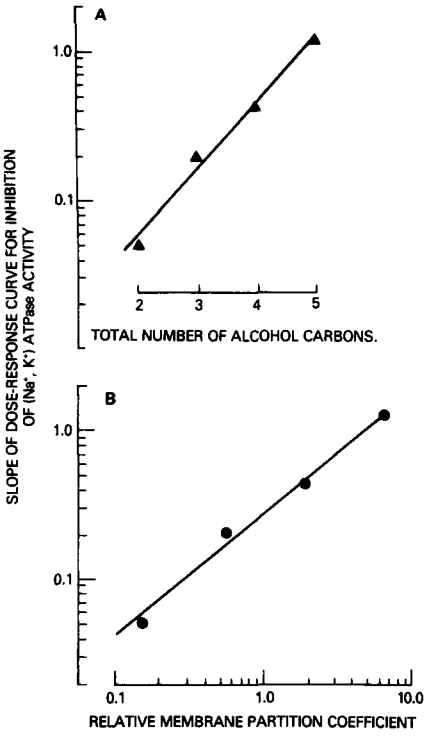

Fig. 1. Correlation between potency of alcohols to inhibit (Na^+,K^+)ATPase and alcohol chain length (A) or relative membrane:water partition coefficients (B). (Na^+,K^+)ATPase activity in mouse synaptosomal membranes was measured in the absence and presence of various concentrations of alcohols, as described in the text. Values on the ordinate represent the logarithm of the slope of the dose–response curve for inhibition of (Na^+,K^+)ATPase activity by each alcohol. Results represent the mean of three separate experiments using each alcohol, in which all values were within 5% of the mean.

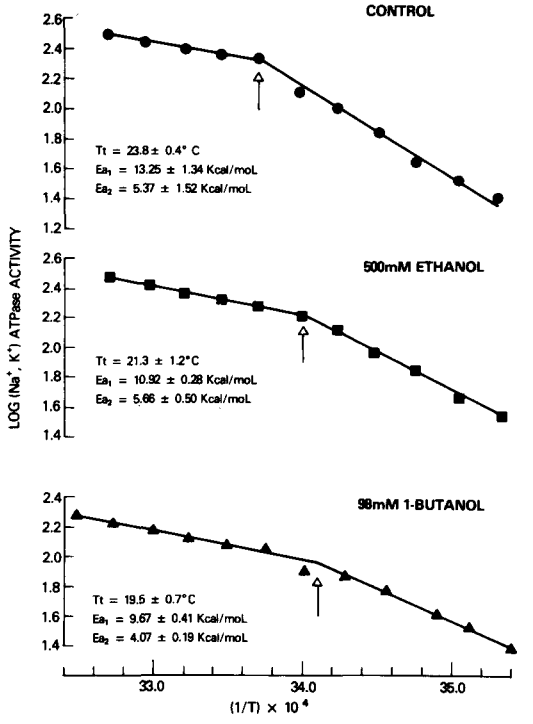

Fig. 2. Temperature dependence of mouse brain (Na^+,K^+)ATPase activity in the absence (●) and presence of 500 mM ethanol (■) or 98 mM 1-butanol (▲). Transition temperatures (↑) and activation energies (Ea_1—below the transition temperature; Ea_2—above the transition temperature) were determined from a computerized analysis of the data, as previously described [1]. Values represent mean from at least four separate experiments, in which all values were within 6% of the mean.

liminary studies, assessments were made of the effects of NE (10^{-9} to 10^{-3} M) on the activity of either the partial (*p*-nitrophenylphosphatase) or whole reaction of (Na^+,K^+)ATPase (in 50 mM MOPS–NH_4) in non-detergent-treated crude synaptosomal membranes of mice. The catecholamine did not cause significant alteration of either activity, and neither vehicle had any effect. Changing the KCl concentration (increasing to 20 mM or decreasing to 1 mM) or the ATP concentration (1 or 3 mM), or reducing NE metabolism by preincubating tissue with pargyline (0.1 mM), an MAO inhibitor, did not enhance the response of (Na^+,K^+)ATPase activity to NE.

Norepinephrine has been reported to potentiate the inhibitory effect of low concentrations of ethanol (50 mM) on rat brain (Na^+,K^+)ATPase activity [4, 13, 14]. In mice, we found that 1 μM NE significantly potentiated the effects of ethanol only when the alcohol was present at a concentration higher than 600 mM (data not shown; assay performed in 50 mM MOPS–NH_4^+ buffer, pH 7.4).

To determine whether the potentiation of the inhibitory effect of ethanol by NE was species-dependent, we compared the combined effects of ethanol and NE on (Na^+,K^+)ATPase activity in mouse and rat brain preparations under the same conditions, which were chosen to be identical to those previously reported [4, 13, 14]. The results shown in Table 1 demonstrate that, while enzyme activity was higher in rat brain preparations than in mouse brain preparations, NE did not cause significant changes in (Na^+,K^+)ATPase activity in mouse or rat brain, and the catecholamine did not potentiate the inhibitory effect of 50 mM ethanol on enzymic activity in brain tissue from either species.

Effect of ethanol on Li^+*-dependent phosphatase activity.* Li^+-dependent phosphatase activity has been reported to be a direct measure of the catalytic activity of (Na^+,K^+)ATPase [10]. Addition of 500 mM ethanol to the assay resulted in significant inhibition of Li^+-dependent activity (Table 2).

DISCUSSION

The activity of mouse brain (Na^+,K^+)ATPase was inhibited by all of the short-chain aliphatic alcohols that were tested, and there was a positive linear relationship between inhibitory potency and both alcohol membrane:water partition coefficient, and alcohol chain length. Similar results were reported for effects of some aliphatic alcohols on K^+-depen-

Table 1. Effects of NE and ethanol on (Na^+,K^+)ATPase in mouse and rat brain

Additions	(Na^+,K^+)ATPase activity (μmoles P_i/mg protein/hr)	
	Mouse synaptosomal preparation	Rat synaptosomal preparation
None	41.4 ± 5.8	53.0 ± 2.6*
1 μM NE	40.6 ± 3.9	52.4 ± 1.6
100 μM NE	41.2 ± 5.1	50.7 ± 3.4
50 mM EtOH	36.7 ± 5.1	49.6 ± 3.8
500 mM EtOH	23.8 ± 2.0†	31.1 ± 2.2†
1 μM NE + 50 mM EtOH	35.8 ± 1.6	48.7 ± 3.2
100 μM NE + 50 mM EtOH	36.1 ± 2.4	49.9 ± 1.5

(Na^+,K^+)ATPase activity was measured in detergent-treated crude synaptic membranes from brains of mice or rats (see text for details). Each value is the mean ± S.D. of four separate experiments.

* In all cases, the enzyme activity was significantly higher in preparations from rat brain relative to those from mouse brain ($P < 0.05$, Student's t-test, df = 6).

† $P < 0.05$ compared to control values (no additions) (Student's t-test, df = 6).

dent p-nitrophenylphosphatase activity in microsomes from bovine brain [12] and on (Na^+, K^+)ATPase activity in synaptic membranes from guinea pig [22] and rat [3] brains. The present data are consistent with the hypothesis that the inhibition of (Na^+,K^+)ATPase by alcohols depends, to a considerable extent, on their ability to partition into cell membranes. However, the ability of alcohols to perturb membrane lipids, once they have partitioned into the membrane, does not appear to account totally for enzyme inhibition. For example, the lipid solubility of the alcohols increases by a factor of 3.5 for each methylene group [21]. When the logarithm of membrane-disordering ability of alcohols was related to their chain length, the slope of the regression line was 0.57, indicating that disordering capacity increased by a factor of 3.7 per methylene group (i.e. $10^{0.57}$), comparable to the lipid solubility factor [21]. Thus, lipid-disordering potency appeared to be directly related to the capacity of alcohols to partition into the membrane. In contrast, the slope of the regression line relating potency of (Na^+,K^+)ATPase inhibition to alcohol chain length was 0.45, indicating that inhibition increased only by a factor of 2.8-fold ($10^{0.45}$) per methylene group. Using the published data [21], we compared the slope of the regression line reported by Lyon *et al.* [21] to the slope of the line calculated from our data, and found that the 90% confidence limits did not overlap. Furthermore, the membrane-disordering potency of these alcohols has been shown to be a direct logarithmic function of the membrane-water partition coefficient [21]. In our study, the slope of the regression line relating inhibitory potency to partition coefficient was less than one, again suggesting that the alcohols are somewhat less efficient in inhibiting (Na^+,K^+)-ATPase activity than in disordering membrane lipids.

Table 2. Effect of LiCl and ethanol on p-nitrophenylphosphatase activity in mouse brain

Additions	p-Nitrophenylphosphatase activity (nmoles p-nitrophenol/mg protein/hr)	
	Control	+500 mM Ethanol
30 mM LiCl	0.18 ± 0.06	0.03 ± 0.01*

p-Nitrophenylphosphatase activity was measured as described in the text. Each value is the mean ± S.D. of three separate experiments.

* $P < 0.05$ compared to value in the absence of ethanol (Student's t-test. df = 4).

The previously reported relationship between membrane disordering potency (measured by EPR) and alcohol : water partition coefficient (slope = 1.0) reflects the ability of the alcohols to disorder bulk membrane lipids [21]. The activity of (Na^+, K^+)ATPase appears to depend primarily on the properties of lipids immediately surrounding the enzyme ("boundary lipids") [23, 24]. If the ability of alcohols to alter the properties of such boundary lipids is not a direct function of the membrane : water partition coefficient, then the apparently low inhibitory potency could in fact indicate that inhibition of enzyme activity is related to effects on boundary lipids or on lipid–protein interactions, rather than to effects on bulk lipids. The transition temperature of (Na^+,K^+)ATPase has been demonstrated to be an indication of the phase transition of lipids surrounding the enzyme [25]. Thus, changes in transition temperature presumably provide an accurate indication of the effects of alcohols on the properties of the boundary lipids of (Na^+,K^+)ATPase. Ethanol, added *in vitro*, has been demonstrated consistently to reduce the transition temperature of (Na^+,K^+)-ATPase in brain membrane preparations [1, 20, 26] although there was one report that ethanol was ineffective in this respect [27]. Our data confirm earlier results [1, 20, 26], and also indicate that identical membrane concentrations of ethanol and butanol cause similar decreases in transition temperature. Therefore, the ability of the alcohols to affect the

properties of the enzyme "boundary" lipids *does* seem to be directly related to the amount of alcohol which partitions into the membrane, and an alternative explanation for the somewhat low inhibitory potency, as related to the partition coefficients, must be considered.

A number of mechanisms have been proposed to account for the inhibitory effect of ethanol on (Na^+, K^+)ATPase activity (e.g. reduced affinity of the regulatory site for K^+ [12]; alteration of conformational changes involved in enzyme activity [8]. (Na^+,K^+)ATPase activity involves the interconversion between two conformations of the enzyme: E_1 (high affinity for ATP) and E_2 (high affinity for K^+) [10]. The rate-limiting step in the overall enzyme reaction may be the transition from E_2 to E_1 (see Ref. 10). Agents which increase membrane fluidity seem to stabilize the enzyme in the E_1 conformation [10, 12] and enhance the transition of E_2 to E_1 [12]. Such an effect would lead to an increased rate of enzyme activity, which does occur when membrane fluidity is increased by changes in temperature or membrane lipid components [10, 11]. However, ethanol as well as fatty acids, which increase membrane fluidity, inhibit, rather than stimulate, ATPase activity [28]. The inhibitory effect of alcohols and fatty acids could be a result of an additional direct effect on the enzyme catalytic site. Based on the observed effects of ethanol on Arrhenius activation energies, Rangaraj and Kalant [20] postulated that inhibition of (Na^+,K^+)ATPase activity by ethanol, at temperatures above the transition temperature, results from a direct interaction of ethanol with the enzyme protein. Similarly, Lin [24] interpreted his results with reconstituted (Na^+,K^+)ATPase to indicate that the inhibitory effect of ethanol involved a direct interaction with the enzyme protein [24].

While most of our experiments did not directly address the mechanism of ethanol's actions, we did investigate the effect of ethanol on Li^+-dependent *p*-nitrophenylphosphatase activity [10]. (Na^+,K^+)-ATPase-associated phosphatase activity is a measure of the activity of the enzyme in the E_2 conformation. Because Li^+ is an effective activator of this activity only at the phosphatase catalytic site, but not at regulatory sites, phosphatase activity in the presence of Li^+ does not depend on enzyme conformational changes which may be influenced by membrane fluidity. Thus, Li^+-dependent phosphatase activity reflects only the catalytic activity of the enzyme in the E_2 conformation [10]. The elimination of Li^+-dependent activity by a high concentration of ethanol (500 mM) may therefore indicate an effect on the phosphatase catalytic site. A similar reduction of Li^+-dependent phosphatase activity can be inferred from the results of Swann [10]. Such findings are consistent with a direct effect of ethanol on the enzyme protein.

There has been controversy with regard to the effect of NE on brain (Na^+,K^+)ATPase activity. The described stimulatory effect on NE on enzymic activity [29–31] has been postulated to be due to chelation by NE of inhibitory divalent metal ions [30] or vanadium [32], present in the commercial preparations of equine muscle ATP. However, other investigators have also reported stimulation of (Na^+,K^+)ATPase activity by catecholamines when the substrate was synthetic ATP containing no vanadium or other known metallic inhibitors [33, 34]. In studies that utilize synthetic ATP (vanadium-free), it is unclear to what extent NE stimulation of (Na^+,K^+)ATPase activity is dependent on the Mg^{2+} : ATP ratio. Desaiah and Ho [34, 35] described stimulation of enzyme activity by catecholamines using a wide range of Mg^{2+} : ATP ratios, while Kalant and Rangaraj [13] reported a stimulatory effect of NE only at high ($> 1:1$) Mg^{2+} : ATP ratios. In contrast, Wu and Phillis [33] observed a stimulatory effect of NE on (Na^+,K^+)ATPase activity at a low Mg^{2+} : ATP ratio. In our experiments, no stimulation by NE was observed at any Mg^{2+} : ATP ratio tested (3:1) and 1:1); similarly Syapin *et al.* [36], using brain tissue of C57Bl mice, recently reported no stimulation of (Na^+,K^+)ATPase activity by NE (Mg^{2+} : ATP ration 1:1). It appears that the factors responsible for the observation of a stimulatory effect of NE on (Na^+,K^+)ATPase activity need further investigation.

Catecholamines have also been reported to enhance the sensitivity of rat brain (Na^+,K^+)ATPase to inhibition by ethanol [4, 13, 14], by a mechanism independent of that associated with activation of (Na^+,K^+)ATPase by catecholamines. Rangaraj and Kalant [4, 13, 14] observed that 50 mM ethanol, a concentration attainable *in vivo* [37], produced significant inhibition of (Na^+,K^+)ATPase activity in the presence of 1 μM (or lower) NE and no significant inhibition in its absence. This finding is important since it suggests that, *in vivo*, ethanol may specifically inhibit (Na^+,K^+)ATPase activity in noradrenergically-innervated brain areas, leading to alterations in neuronal activity in these regions. In the present study, we observed a small, non-significant inhibition of (Na^+,K^+)ATPase activity by 50 mM ethanol in preparations from both mouse and rat brain (11 and 12%, respectively, Table 1). However, we did not observe any potentiation by either 1 or 100 μM NE of the inhibitory effect of 50 mM ethanol. The lack of sensitization by NE is probably not a result of loss of cellular elements necessary for the action of NE, due to preparation of brain tissue in detergent. Rangaraj and Kalant [4, 13, 14], using an identical brain preparation, observed increased inhibition by ethanol in the presence of NE. In particular, Kalant and his colleagues provided evidence that sensitization to ethanol inhibition by catecholamines was mediated by α-adrenergic receptors [4, 13, 14], indicating the presence of catecholamine binding sites in these brain preparations. At this time, we have no explanation for the differences between our results and those of Kalant and his colleagues, since we used the same species and strains of animals, and identical assay conditions to those reported previously [4, 13, 14]. It is of interest that Syapin *et al.* [36], using brain tissue from C57Bl mice, also reported that NE does not potentiate inhibition of (Na^+, K^+)ATPase activity by ethanol. However, it is clear that a number of variables, not all of which have as yet been systematically analyzed, can influence the response to NE.

Overall, our results support the hypothesis that ethanol can affect (Na^+,K^+)ATPase activity by par-

titioning into the membrane lipids surrounding the enzyme, and possibly by acting directly on the enzyme protein. Although high concentrations of ethanol can inhibit brain (Na^+,K^+)ATPase activity, and resistance to this inhibition occurs after chronic ethanol treatment [1], the role of inhibition of (Na^+, K^+)ATPase in mediating the effects of ethanol in the CNS *in vivo* remains open to question since, under our conditions, concentrations of ethanol attainable *in vivo* had only a slight, non-significant inhibitory effect on the enzyme.

Acknowledgements—This work was supported in part by a grant from ADAMHA, PHS (AA 2696).

REFERENCES

1. M. Levental and B. Tabakoff, *J. Pharmac. exp. Ther.* **212**, 315 (1980).
2. D. B. Goldstein and Y. Israel, *Life Sci.* **11**, 957 (1972).
3. Y. Israel, H. Kalant and A. E. LeBlanc, *Biochem. J.* **100**, 27 (1966).
4. N. Rangaraj and H. Kalant, *Can. J. Physiol. Pharmac.* **57**, 1098 (1979).
5. H. K. Kimelberg, S. Biddlecome, S. Narumi and R. S. Bourke, *Brain Res.* **141**, 305 (1978).
6. B. Dekruyff, P. W. M. Van Dijck, R. W. Goldbach, R. A. Demel and L. L. M. Van Deenen, *Biochim. biophys. Acta* **330**, 269 (1973).
7. J. H. Chin and D. B. Goldstein, *Science* **196**, 684 (1977).
8. H. Kalant, N. Woo and L. Endrenyi, *Biochem. Pharmac.* **27**, 1353 (1978).
9. P. Seeman, *Pharmac. Rev.* **24**, 583 (1972).
10. A. C. Swann, *Archs Biochem. Biophys.* **221**, 148 (1983).
11. F. Giraud, M. Claret, K. R. Bruckdorfer and B. Chailley, *Biochim. biophys. Acta* **647**, 249 (1981).
12. A. C. Swann, *J. biol. Chem.* **258**, 11780 (1983).
13. H. Kalant and N. Rangaraj, *Eur. J. Pharmac.* **70**, 157 (1981).
14. N. Rangaraj and H. Kalant, *Can. J. Physiol. Pharmac.* **58**, 1342 (1980).
15. C. W. Cotman, *Meth. Enzym.* **31**, 445 (1974).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
17. M. Kurokawa, M. Kato and T. Sakamoto, *Biochem. J.* **97**, 833 (1965).
18. R. Penniall, *Analyt. Biochem.* **14**, 87 (1966).
19. A. C. Swann, J. L. Marini, M. H. Sheard and J. W. Maas, *Biochem. Pharmac.* **29**, 2819 (1980).
20. N. Rangaraj and H. Kalant, *J. Pharmac. exp. Ther.* **223**, 536 (1982).
21. R. C. Lyon, J. A. McComb, J. Schreurs and D. B. Goldstein, *J. Pharmac. exp. Ther.* **218**, 669 (1981).
22. A. Y. Sun and T. Samorajski, *J. Neurochem.* **17**, 1365 (1970).
23. H. K. Kimelberg and D. Papahadjopoulos, *Biochim. biophys. Acta* **282**, 277 (1972).
24. D. C. Lin, *Biochem. Pharmac.* **29**, 771 (1980).
25. J. S. Charnock and C. L. Bashford, *Molec. Pharmac.* **11**, 766 (1975).
26. C. Guerri and S. Grisolia, *Pharmac. Biochem. Behav.* **18**, (Suppl. 1), 45 (1983).
27. J. Y. Westcott and H. Weiner, *Archs Biochem. Biophys.* **223**, 51 (1983).
28. A. C. Swann, *Archs Biochem. Biophys.* **233**, 354 (1984).
29. K. Yoshimura, *J. Biochem. Tokyo.*, **74**, 389 (1973).
30. T. D. Hexum, *Biochem. Pharmac.* **26**, 1221 (1977).
31. S. L. Lee and J. W. Phillis, *Can. J. Physiol. Pharmac.* **55**, 961 (1977).
32. L. C. Cantley, Jr., L. Josephson, R. Warner, M. Yanagisawa, C. Lechene and G. Guidotti, *J. biol. Chem.* **252**, 7421 (1977).
33. P. H. Wu and J. W. Phillis, *Gen. Pharmac.* **9**, 421 (1978).
34. D. Desaiah and I. K. Ho, *J. Pharmac. exp. Ther.* **208**, 80 (1979).
35. D. Desaiah and I. K. Ho, *Biochem. Pharmac.* **26**, 2029 (1977).
36. P. J. Syapin, J. Chen and R. L. Alkana, *Alcohol* **2**, 145 (1985).
37. H. Kalant, in *The Biology of Alcoholism* (Eds. B. Kissin and H. Begleiter), Vol. 1, p. 1. Plenum Press, New York (1971).