Biochemical Pharmacology, Vol. 27, pp. 1139–1144.

0006-2952/78/0415-1139$02.00/0

EFFECTS OF ETHANOL WITHDRAWAL, STRESS AND AMPHETAMINE ON RAT BRAIN ($Na^+ + K^+$)-ATPase

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(*Received* 10 *June* 1977; *accepted* 8 *September* 1977)

Abstract—Rats were fed liquid diets containing ethanol (EtOH) or sucrose for 4 weeks, and killed at various times after removal of EtOH. In those having access to the diet up to the time of sacrifice, EtOH caused no increase in ($Na^+ + K^+$)-ATPase activity of whole brain homogenates. However, activity was increased during the period 12–48 hr after withdrawal of EtOH, and was greatest at 24 hr. Fractionation of the homogenate showed that the increase was confined to the lysed synaptosomal fraction. Activity was also increased at 16 hr after one acute dose of EtOH (5 g/kg). The increased ATPase activity during withdrawal could be blocked by administration of another dose of EtOH (3 g/kg) 1 hr before sacrifice, in both acutely and chronically EtOH-treated rats. This is consistent with enhanced sensitivity of noradrenaline-stimulated enzyme to inhibition by EtOH *in vitro*. ATPase activity was also increased by amphetamine in a dose-dependent manner, both *in vivo* and *in vitro*, and by forced swimming. The rise in ($Na^+ + K^+$)-ATPase activity during EtOH withdrawal is interpreted as an activation by conformational change, secondary to catecholamine release due to stress, rather than an adaptive response to chronic EtOH exposure.

($Na^+ + K^+$)-ATPase (EC 3.6.1.3) is believed to mediate active transport of monovalent cations across cell membranes and is involved in maintenance of normal resting membrane polarization (1). The enzyme activity is inhibited by ethanol (EtOH) *in vitro* [2, 3] and this has been confirmed by many investigators [4–8]. In contrast, the effect of chronic treatment with EtOH on brain ($Na^+ + K^+$)-ATPase is variable.

The enzyme activity has been reported to be increased in homogenates of rat brain cerebral cortex and medulla oblongata [4, 9], and of frontal and association cortex and hippocampus of cats [10], upon withdrawal after chronic EtOH treatment. The increased enzyme activity has been considered a biochemical correlate of alcohol tolerance and physical dependence [9]. Severity of withdrawal reaction has been correlated with the increased ($Na^+ + K^+$)-ATPase activity in rat brain crude mitochondrial and microsomal fractions [11, 12]. On the other hand, no change in activity has been observed in brain cortex of physically dependent mice [5], microsomal fractions from rat brain [4], homogenates of rat midbrain and cerebellum [4], and caudate nucleus, reticular formation and amygdala from cat brain [10].

Noradrenaline (NA), dopamine (DA) and serotonin (5HT) have been reported to activate ($Na^+ + K^+$)-ATPase in homogenates of whole mouse brain [13], rat hypothalamus and cerebral cortex [14] and cat cerebral cortex [15]. Logan and O'Donovan [16] found that 10^{-5} M NA or 5HT stimulated the enzyme in rabbit brain cortex synaptosomal plasma membranes (SPM) by 100 per cent, but NA, 5HT and DA did not have a stimulatory effect of SPM isolated from other regions of brain, such as striatum, corpora quadrigemina, midbrain, cerebellum and brain stem. Recently Jeffrey and Gibbs [17] have reported that NA, but not *d*-amphetamine, stimulated the enzyme from chicken forebrain homogenates. On further fractionation they found that the chicken forebrain synaptosomal ($Na^+ + K^+$)-ATPase was increased 35 per cent by NA (10^{-4} M) and *d*-amphetamine (10^{-5} M), while amphetamine at 10^{-2} M inhibited the enzyme by 30 per cent.

Studies on the role of neurotransmitters in physical dependence are controversial [18]. NA turnover has been found to increase during EtOH withdrawal, the increase possibly being secondary to the withdrawal reaction [19–21]. The increased ($Na^+ + K^+$)-ATPase activity after chronic exposure to EtOH and withdrawal has been attributed to an adaptive mechanism compensating for stress [22].

If increased ATPase activity is a manifestation or consequence of the stress or hyperactivity associated with an EtOH withdrawal reaction, rather than an adaptive response to the chronic effects of EtOH, the increase should show the same time course as the withdrawal reaction and should be reversible by further administration of EtOH. In addition, comparable degrees of stress or hyperactivity produced by other means should give comparable increases in ATPase activity. Therefore, we have studied the ATPase activity in brain preparations from rats at various times after acute and chronic administration of EtOH, after acute treatment with amphetamine, and after hyperactivity in the form of forced swimming.

MATERIAL AND METHODS

Male Wistar rats from Biobreeding Laboratories (Ottawa, Canada) were individually housed in temperature-controlled rooms. For the chronic

EtOH experiments, rats weighing 150–175 g were fed liquid diets [23] providing 36 per cent of calories as EtOH, while the controls were pair-fed an equivalent diet in which EtOH was equicalorically replaced by sucrose. After 4–5 weeks of pair feeding some animals from each group were kept on diet until sacrificed. Other rats were sacrificed at 12, 24 and 48 hr after the last EtOH feeding; in these cases, both EtOH and control animals received only water for the final 12 or 24 hr.

The animals were decapitated, blood was collected for EtOH determination [24], and the brain was removed as quickly as possible, wiped clean of blood, weighed and homogenized at low speed in 10 vol. of ice-cold 0.32 M sucrose in a glass Potter–Elvehjem homogenizer with Teflon pestle. An additional 10 vol. of sucrose solution used to rinse the homogenizer was added to the homogenate.

Synaptosomes were prepared from whole brain homogenates according to the method of Gurd *et al.* [25]. The crude mitochondrial pellet (P_2) after three washings was resuspended in 0.32 M sucrose and layered onto a discontinuous gradient of 7.5% Ficoll and 13% Ficoll dissolved in 0.32 M sucrose buffered with 5 mM Hepes at pH 7.6. After centrifugation for 1.5 hr at 105,000 *g*, the synaptosomal fraction was removed from the interface between the 7.5 and 13% Ficoll layers, diluted with 3 vol of 0.32 M sucrose and centrifuged at 35,000 *g* for 30 min. The synaptosomal fraction was resuspended in 0.32 M sucrose and lysed with 5 mM Hepes at pH 8.4 for 90 min at 4°. Again the synaptosomal particulate fraction (lysed synaptosomes) was pelleted down by centrifuging at 35,000 *g* for 30 min.

The fraction above the 7.5% Ficoll layer (myelin fraction) was also diluted with 3 vol of 0.32 M sucrose and centrifuged at 35,000 *g* for 30 min. The myelin fraction was resuspended in 0.32 M sucrose. The fraction at the bottom of the 13% Ficoll layer (mitochondrial fraction) was washed once by suspending in 0.32 M sucrose and centrifuging at 35,000 *g* for 30 min. The mitochondrial pellet was suspended in 0.32 M sucrose.

Acute ethanol treatment. Male Wistar rats weighing 260–300 g were given by gastric intubation 5 g/kg of EtOH as a 20% (w/v) solution in water, and the weight-matched control rats were given an equal volume of equicaloric sucrose solution. Water and chow diet were freely available at all times. Sixteen hr after treatment, the animals were sacrificed and brain homogenates in 0.32 M sucrose were prepared as described above.

Some acutely treated rats were given a second dose of EtOH (3 g/kg) 15.5 hr after the first dose, and were sacrificed 1 hr later. Some chronically EtOH-treated rats after 12 hr of withdrawal were also given an acute dose of EtOH (3 g/kg) 1 hr before sacrifice. Brain homogenates were prepared.

Stress by swimming. Ten rats weighing 220–280 g were made to swim in a tank of water at 17–19°. Two-min swim periods alternated with two-min rest periods for a total of 45–60 min. Immediately after the last swim animals were sacrificed, and the brain was removed and homogenized as described above. Ten weight-matched control rats were used for comparison, but one homogenate was lost.

Amphetamine experiments. Rats weighing 220–300 g were given different doses of *d*-amphetamine (5–20 g/kg, i.p.) in saline. Control rats were given only saline by the same route. After exactly 1 hr the animals were decapitated and brain homogenates were prepared.

$(Na^+ + K^+)$-ATPase assay. Homogenates and subcellular fractions were diluted appropriately either 5- or 10-fold with cold distilled water, and assays were done on 0.05 ml- and 0.1 ml-samples of each, to verify proportionality of activity. The assay mixture contained 30 mM imidazole, 30 mM glycylglycine, 4 mM $MgCl_2$, 4.5 mM ATP, 120 mM NaCl and 5 mM KCl plus enzyme in a final volume of 1.2 ml at pH 7.4. In another tube, 10^{-3} M ouabain was added, and NaCl and KCl were omitted [26]. Reaction was started by adding the enzyme, and the mixture was incubated for 20 min at 37° in a shaking water bath [26]. Previous studies in this laboratory have shown the reaction to be linear for at least 30 min. The reaction was stopped by adding 0.5 ml of 10% $HClO_4$. Inorganic phosphate (P_i) was estimated by the phosphomolybdate method as modified by Post and Sen [26].

$(Na^+ + K^+)$-ATPase activity was obtained by subtracting the ouabain-insensitive activity (Mg^{2+}-ATPase) from the total activity in the presence of Na^+, K^+ and Mg^{2+} and the absence of ouabain. The activity was expressed as μmoles P_i produced/mg of protein × hr or μmoles P_i produced/g of brain × 20 min. In some experiments, the K^+ concentration was varied from 0.5 to 18.5 mM, with and without the addition of 0.05 M EtOH and 0.1 mM NA, and the apparent K_m for K^+ was obtained from Lineweaver–Burk transformation of the results.

Blood ethanol measurement. Blood EtOH was determined enzymatically according to the method described by Hawkins *et al.* [24]. Protein was determined by the method of Lowry *et al.* [27] using bovine serum albumin as standard.

RESULTS

Effect of acute and chronic ethanol administration and withdrawal on $(Na^+ + K^+)$-ATPase. When rats were given one acute dose of 5 g/kg of EtOH, a 16 per cent increase in specific activity of $(Na^+ + K^+)$-ATPase was observed in brain homogenates prepared 16 hr later (Table 1). This increased activity could be blocked by a second dose of EtOH (3 g/kg) administered 1 hr before sacrifice. The protein content of the brain did not change, suggesting that there was no increased enzyme synthesis. The activity per g of brain was slightly but significantly reduced below control levels by a second dose of EtOH.

Rats on the liquid diets (Table 2) grew approximately equally in the EtOH and sucrose groups. The specific activity of $(Na^+ + K^+)$-ATPase did not increase in chronically EtOH-treated rats when the animals were kept on the EtOH liquid diet until the time of sacrifice. The maximum blood EtOH levels on such diets occur during the night when most of the rat's food ingestion takes place [28]. The blood EtOH concentration at sacrifice (10:00 a.m.) was still fairly high, 113 ± 20.64 mg/100 ml (mean ± S.

Table 1. Brain $(Na^+ + K^+)$-ATPase activity and protein 16 hr after an acute dose (5 g/kg) of ethanol*

	$(Na^+ + K^+)$-ATPase (μmoles P_i/mg protein × hr)	Activity/g brain × 20 min	Protein (mg/g brain)	Brain wt (g/100 g body wt)
Without a second dose				
Sucrose	13.95 ± 0.12 (5)	629.2 ± 13.5	135.32 ± 2.65	0.578 ± 0.036
Ethanol	16.26 ± 0.43† (6)	714.0 ± 15.5†	131.82 ± 3.06	0.590 ± 0.043
With a second dose‡				
Sucrose	14.16 ± 0.33 (5)	637.7 ± 11.5	135.34 ± 3.73	0.590 ± 0.029
Ethanol	13.75 ± 0.43 (6)	618.8 ± 9.9§	135.10 ± 4.00	0.584 ± 0.026

* Values are reported as mean ± S. D.; the number of animals is shown in parentheses.
† $P < 0.001$.
‡ A second dose of ethanol (3 g/kg) was given to acutely ethanol-treated rats and equicaloric sucrose given to control rats (sucrose) 1 hr before sacrifice.
§ $P < 0.02$.

D.). In contrast, an increase of 13–15 per cent in activity was observed in brain homogenates from rats withdrawn from EtOH for 12–48 hr (Table 2). There was a significant decrease in protein content after chronic EtOH treatment. Since the rats were pair-fed and the analyses on the pair-mates were done at the same time, statistical analysis was done by Student's 't' test for paired data. The difference, though small, was consistent. The lower protein content was not accompanied by higher brain weight in the EtOH-treated rats. The increased $(Na^+ + K^+)$-ATPase observed after withdrawal is in agreement with some of the earlier reports [9–12, 22]. An acute dose of EtOH (3 g/kg) given to 12-hr withdrawn rats, 1 hr prior to sacrifice, blocked the stimulation of enzyme activity (Table 2).

The subcellular distribution of the enzyme activity is shown in Table 3. The specific activity of $(Na^+ + K^+)$-ATPase did not increase in any of the fractions isolated from non-withdrawn rats nor in most of the fractions from withdrawn rats. There was an increased activity in synaptosomal and lysed synaptosomal fractions from 24- and 48-hr withdrawn rats. The lysed synaptosomes of 24-hr withdrawn rats showed a 28 per cent increase, whereas in 48-hr withdrawn rats the enhancement was 16 per cent. This indicates that the withdrawal effect is disappearing and the activity is approaching normal values. However, whole brain homogenates from withdrawn rats showed equal stimulation after different withdrawal times (12–48 hr).

Addition of 0.05 M EtOH to rat brain homogenates had no effect on $(Na^+ + K^+)$-ATPase activity. In contrast, addition of 0.1 mM NA stimulated the activity significantly; for example, the increase was about 35 per cent at 5 mM K^+ ($P < 0.01$). A Lineweaver–Burk plot (Fig. 1) showed no change in apparent K_m for K^+ by either EtOH or NA alone.

Table 2. Effect of chronic ethanol treatment and withdrawal on brain protein and $(Na^+ + K^+)$-ATPase*

Treatment	Body wt (g)	Brain wt (g/100 g body wt)	Protein content (mg/g brain)	$(Na^+ + K^+)$-ATPase (μmoles P_i/mg protein × hr)
Non-withdrawn				
Sucrose (10)	345 ± 28	0.491 ± 0.044	124.8 ± 8.2	13.98 ± 0.51
Ethanol (10)	363 ± 40	0.475 ± 0.053	116.6 ± 9.7†	13.68 ± 1.00
12-hr Withdrawn				
Sucrose (6)	375 ± 41	0.442 ± 0.049	141.3 ± 3.8	13.79 ± 0.48
Ethanol (6)	382 ± 29	0.448 ± 0.024	137.2 ± 4.0‡	15.64 ± 0.48†
12-hr Withdrawn + acute dose§				
Sucrose (6)	355 ± 27	0.474 ± 0.054	142.9 ± 2.5	14.18 ± 0.48
Ethanol (6)	360 ± 20	0.484 ± 0.047	136.3 ± 4.9‡	13.88 ± 0.36
24-hr Withdrawn				
Sucrose (10)	331 ± 36	0.511 ± 0.070	136.4 ± 12.4	14.61 ± 0.70
Ethanol (10)	331 ± 41	0.508 ± 0.065	119.4 ± 12.1‡	16.85 ± 0.72†
48-hr Withdrawn				
Sucrose (10)	322 ± 35	0.537 ± 0.074	126.4 ± 6.7	13.77 ± 1.05
Ethanol (10)	328 ± 43	0.510 ± 0.079‖	121.2 ± 10.2‖	15.60 ± 0.41†

* Values are reported as mean ± S. D. Number of animals per group is shown in parentheses. P values were determined by Student's t-test for paired data.
† $P < 0.001$.
‡ $P < 0.01$.
§ An acute dose of ethanol (3 g/kg) was given by intragastric intubation to some of the 12-hr withdrawn rats. Sucrose control rats received an acute dose of equicaloric sucrose. All animals were sacrificed 1 hr later.
‖ $P < 0.05$.

Table 3. Effect of chronic ethanol treatment and withdrawal on $(Na^+ + K^+)$-ATPase in brain subcellular fractions

Preparations	$(Na^+ + K^+)$-ATPase (μmoles P_i produced/mg protein × hr ± S. D.) Non-withdrawn (N = 6) Sucrose	Non-withdrawn (N = 6) Ethanol	24-hr Withdrawn (N = 6) Sucrose	24-hr Withdrawn (N = 6) Ethanol	48-hr Withdrawn (N = 5) Sucrose	48-hr Withdrawn (N = 5) Ethanol
Homogenate	13.85 ± 0.42	13.53 ± 0.93	14.96 ± 0.65	17.13 ± 0.65*	13.75 ± 1.41	15.69 ± 0.23†
Nuclear fraction	12.62 ± 2.57	11.19 ± 2.77	14.64 ± 1.84	16.46 ± 2.06	12.75 ± 2.02	13.86 ± 1.49
Crude mitochondrial (P_2) fraction	19.44 ± 2.18	17.39 ± 3.27	20.12 ± 2.88	21.44 ± 2.97	18.56 ± 2.77	20.27 ± 2.47
Microsomes + supernatant	5.79 ± 0.85	5.86 ± 0.83	6.81 ± 1.30	7.42 ± 1.46	7.20 ± 2.25	7.04 ± 1.19
Myelin	6.13 ± 0.85	7.30 ± 0.82	6.76 ± 0.51	6.93 ± 1.40	5.95 ± 0.60	5.77 ± 0.82
Synaptosomes	20.76 ± 2.14	19.75 ± 2.52	23.66 ± 2.88	28.50 ± 2.62†	23.76 ± 3.25	27.46 ± 3.11‡
Mitochondrial pellet	6.32 ± 0.74	5.97 ± 1.13	7.25 ± 1.19	6.73 ± 0.85	7.21 ± 0.72	6.74 ± 0.62
Lysed synaptosomes	34.84 ± 4.14	33.00 ± 4.55	35.20 ± 1.63	45.07 ± 2.45*	34.10 ± 2.02	39.66 ± 3.89§

* $P < 0.001$.
† $P < 0.02$.
‡ $P < 0.05$.
§ $P < 0.01$.

K_m was approximated from the linear regression line computed from the points at all but the lowest K^+ concentration, at which marked curvilinearity became evident. However, when both 0.1 mM NA and 0.05 M EtOH were added, the activity was decreased by 30 per cent ($P < 0.001$), and there was a dramatic rise in K_m for K^+ from 2 to 7.35 mM.

Effect of forced swimming on $(Na^+ + K^+)$-ATPase. After stress created by swimming in water at 17–19°, the $(Na^+ + K^+)$-ATPase activity increased from 15.15 ± 1.57 to 18.02 ± 0.7 ($P < 0.01$). The increase in activity after forced swimming (about 20 per cent) is comparable to acute and chronic ethanol withdrawal effect. The protein content of brain did not change.

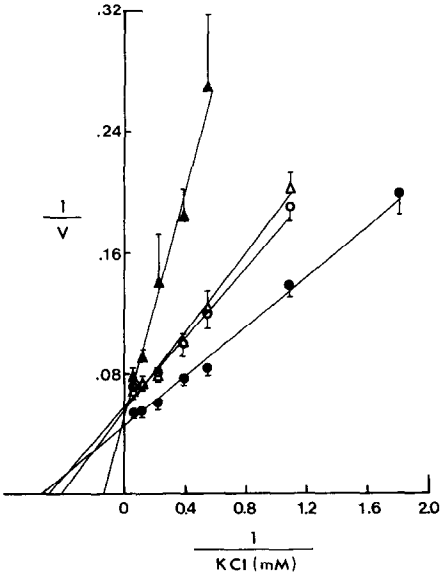

Fig. 1. Lineweaver–Burk plot of the effect of ethanol (0.05 M) and of noradrenaline (0.1 mM), separately and together, on $(Na^+ + K^+)$-ATPase activity of rat brain homogenates in the presence of 120 mM Na^+ and varied concentrations of K^+. V is the velocity of reaction expressed as μmoles P_i produced/mg of protein × hr. Key: controls, ○; EtOH, △; NA, ●; and EtOH+NA, ▲. N = four per point; vertical bars indicate S. E. M. for each point. Regression lines were calculated by the method of least mean squares.

Effect of d-*amphetamine on brain $(Na^+ + K^+)$-ATPase.* The doses of *d*-amphetamine used in this experiment produced the expected hyperactivity and stereotype of behavior. The degree of hyperactivity was not quantified, but the rats showed clear manifestations of amphetamine effect, including hypermotility alternating with complete immobility, markedly exaggerated startle response, piloerection and very aggressive biting responses even to inanimate objects. $(Na^+ + K^+)$-ATPase activity was increased in a dose-dependent fashion with doses of 5–20 mg/kg. The maximum stimulation of 35 per cent was observed at both 15 and 20 mg/kg (Fig. 2). The protein content of the brain was not affected in the present work, ranging from 136.4 ± 4.8 to 139.0 ± 4.2 mg/g in all the treatment groups.

Addition of *d*-amphetamine to rat brain homogenates caused a biphasic effect on $(Na^+ + K^+)$-

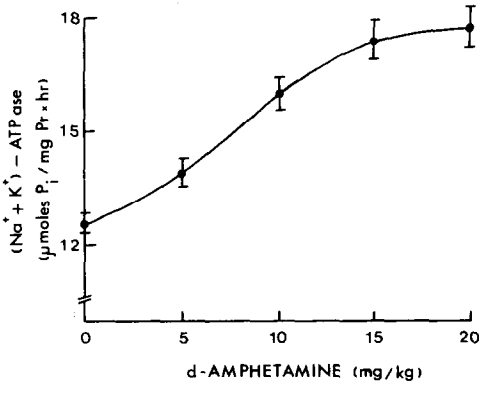

Fig. 2. Effect *in vivo* of *d*-amphetamine on brain $(Na^+ + K^+)$-ATPase. One hr after administration of *d*-amphetamine (5–20 mg/kg, i.p.) brain homogenates were assayed for $(Na^+ + K^+)$-ATPase. Values of N were sixteen for controls and eight for each dose of amphetamine. Vertical bars represent S. E. M. for each dose.

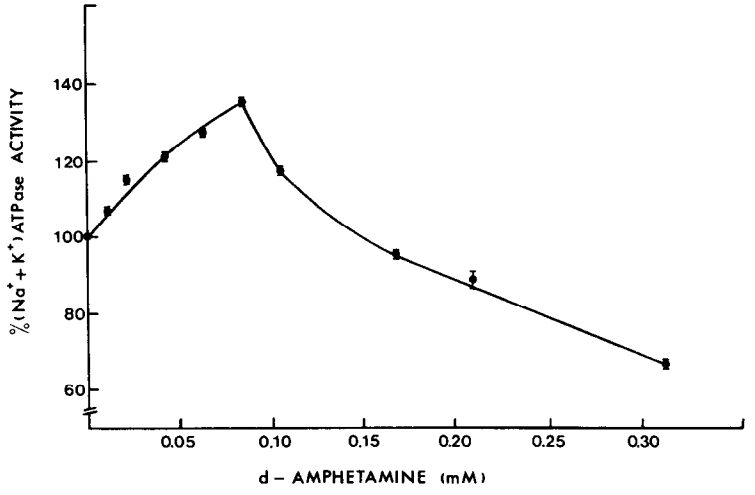

Fig. 3. Effect of various concentrations of *d*-amphetamine added *in vitro* on the activity of $(Na^+ + K^+)$-ATPase in rat brain homogenates. Each point represents at least four determinations. Vertical bars indicate S. E. M. for each point. The values of the ATPase activity at the various concentrations of amphetamine are expressed as percentage of the control values for the respective preparations.

ATPase activity (Fig. 3). Again a maximum stimulation of 35 per cent was observed at 8×10^{-5} M *d*-amphetamine and the activity was 35 per cent below control value at 3×10^{-4} M.

DISCUSSION

The results demonstrate increased $(Na^+ + K^+)$-ATPase activity in brain homogenates, and especially in the lysed synaptosome fraction, during withdrawal from either acute or chronic EtOH treatment. Since none of the brain fractions showed stimulation in non-withdrawn rats, the increase found in synaptosomes and lysed synaptosomes of withdrawn rats is presumably due to post-withdrawal stimulation of the enzyme rather than to an adaptive mechanism compensating for chronic EtOH intoxication.

Our results do not indicate whether the increase in enzyme activity is due to net increase in the enzyme synthesis, or to activation of existing enzyme during withdrawal. The protein content of brain was unchanged after acute EtOH administration but slightly decreased in chronically EtOH-treated rats. This decrease was seen regardless of whether the rats had undergone EtOH withdrawal, indicating that it is a chronic EtOH treatment effect rather than a withdrawal effect. It was apparently not due to edema, since the brain weight was not consistently increased relative to body weight, nor was there a consistent inverse relation between brain weight and protein content (Table 2). The decreased protein content is consistent with reports of decreased brain protein synthesis [29] and of disappearance of a specific protein of mol. wt 210,000–215,000 after chronic EtOH treatment [30]. These findings do not prove that there is no new ATPase synthesis, since net increase of one enzyme may not measurably affect total protein.

NA, DA and 5HT have been shown to activate brain $(Na^+ + K^+)$-ATPase *in vitro* [13–16], and indirect evidence suggests that NA and 5-HT do so *in vivo* [31]. During EtOH withdrawal, NA turnover has been shown to increase [20, 21], whereas 5HT levels and turnover are unaffected or decreased [32, 33]. These findings suggest that the increased enzyme activity is the result of NA stimulation during the withdrawal reaction, rather than of chronic EtOH administration *per se*.

The increased turnover of NA in withdrawn rats is suppressed by an acute dose of EtOH [21]. This might explain the suppression of increase in $(Na^+ + K^+)$-ATPase activity in EtOH-withdrawn rats by another dose of EtOH 1 hr before sacrifice (Table 1 and 2). However, an alternative explanation is suggested by the finding (Fig. 1) that the NA-stimulated enzyme shows a greatly increased sensitivity to inhibition by EtOH. This also raises, but does not resolve, the possibility that lack of apparent stimulation after chronic EtOH without withdrawal is really due to interaction between increased release of NA and continued presence of EtOH. Similar interaction with other transmitters is currently under investigation.

Amphetamine is known to release NA and DA from the respective nerve terminals [34]. DA has been shown to have no stimulatory effect on $(Na^+ + K^+)$-ATPase of SPM from various parts of the rabbit brain [15]. However, other investigators have found stimulation by DA in cruder preparations from various species [13, 14, 35, 36]. Experiments with *d*-amphetamine showed a biphasic effect on the enzyme *in vitro*. At low drug concentrations (< 0.1 mM) the enzyme activity was stimulated and at high concentrations (> 0.17 mM) was inhibited (Fig. 3). Tarve *et al.* [37] have also observed an inhibitory effect of amphetamine at 1–20 mM, but they did not test lower concentrations. They observed a biphasic effect *in vivo*, with stimulation at a dose of 5 mg/kg and inhibition at 20 mg/kg. In our experiments, the increase *in vivo* in ATPase showed a plateau in the dose range of 15–20 mg/kg; possibly a higher dose might have caused a reduction.

Recently Jeffrey and Gibbs [17] also found biphasic effects of *d*-amphetamine, but only with synaptosomes and not with homogenates of chicken

forebrain. They attribute the lack of stimulatory effect in homogenates to the use of hypotonic conditions (homogenization in water) which free all of the stored NA and thus stimulate the enzyme maximally. In contrast, the preparation of synaptosomes in isotonic sucrose does not release stored NA and this, in their view, leaves the capability of response to amphetamine. We used isotonic sucrose for homogenization but diluted it 10-fold with water before use and still observed activation. Moreover, the concentration of NA in brain is about 3 nmoles/g, according to various authors [38, 39], and this would yield a final concentration of only about 0.5 nM in our incubation mixtures. Even if amphetamine released all of this, it would be substantially less than the threshold concentration of 0.1 to 1 μM added NA required for activation of the ATPase [35]. However, endogenous NA released at the synapse might be more effective than exogenous NA. Therefore, it is conceivable that stimulation of the enzyme activity is due to catecholamines, but a direct effect of amphetamine cannot be excluded.

Forced swimming by rats increased brain $(Na^+ + K^+)$-ATPase activity by 20 per cent. Barchas and Freedman [38] observed that NA levels in rat brain decreased after swimming and gradually returned to normal values after 4–6 hr. These findings are consistent with ATPase activation by increased NA release during swimming.

Of the published studies reporting increased $(Na^+ + K^+)$-ATPase after chronic EtOH treatment, all but one were in animals undergoing various degrees of withdrawal reaction [9–12]. However, one study [5] found no increase in mice at the time of peak reaction, 10 hr after ethanol withdrawal. One possible explanation might be a shorter time course of increased NA response in mice than in rats during withdrawal, but this requires an experimental test of NA turnover rates as well as of receptor sensitivity. Sun *et al.* [40] reported a 40 per cent increase in $(Na^+ + K^+)$-ATPase of SPM from dog brain only 2 hr after the last of 21 daily intubations with ethanol. So far, no explanation of the divergence between this finding and the others cited is apparent.

From the available data, it appears probable that increased $(Na^+ + K^+)$-ATPase activity in SPM is a rapid response to increased catecholaminergic activity due to various causes, including ethanol withdrawal. It would appear to be a rapidly reversible activation, consistent with the suggestion of a conformational change in the enzyme [40].

Acknowledgement—The authors are indebted to Mr. B. Oporto, Mr. G. Shah, Mr. G. Ho and Mrs. A. Chau for technical assistance.

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