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EFFECTS OF ALDEHYDES ON SODIUM PLUS POTASSIUM ION-STIMULATED ADENOSINE TRIPHOSPHATASE OF MOUSE BRAIN*

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Abstract—The biogenic aldehydes, 3,4-dihydroxyphenylglycolaldehyde and 3,4-dihydroxyphenylacetaldehyde, derived from norepinephrine and dopamine, respectively, as well as acetaldehyde, porpionaldehyde, benzaldehyde and phenylacetaldehyde, inhibited both $Na^+ + K^+$-activated ATPase and Mg^{2+}-ATPase. In addition, K^+ ion-dependent *p*-nitrophenylphosphatase activity was inhibited by these compounds. The $Na^+ + K^+$-ATPase was much more sensitive than Mg^{2+}-ATPase of K^+-activated phosphatase to inhibition by various aldehydes. The inhibition of $Na^+ + K^+$-ATPase by aldehydes was reversible and was non-competitive with ATP or K^+ as the variable substrate or activator respectively. Addition of cysteine or mercaptoethanol protected the enzymes from inhibition by aldehydes. The concentrations of aldehydes which produced marked inhibition of $Na^+ + K^+$-ATPase ranged from 2×10^{-2} M to 6×10^{-6} M for acetaldehyde and 3,4-dihydroxyphenylglycoladehyde respectively. All aldehydes, including acetaldehyde, were more potent inhibitors of $Na^+ + K^+$-ATPase activity than was ethanol.

The role of the membrane-bound enzyme, Na^+- and K^+-activated, Mg^{2+}-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3), hereafter referred to as $Na^+ + K^+$-ATPase, is well acknowledged. Investigators have observed the activity of this enzyme in a variety of cell membrane fractions from brain, including microsomes and synaptosomes [1]. Several studies have shown that ethanol inhibits $Na^+ + K^+$-ATPase in rat and guinea pig brain microsomes [2, 3], beef brain microsomes [4] and nerve ending fractions from guinea pig cerebral cortex [5]. Although it has been suggested that ethanol is competitive with K^+ ion [2, 3], it is still unclear how ethanol inhibits $Na^+ + K^+$-ATPase. It has recently been reported that acetaldehyde inhibited mitochondrial ATP–Pi^{32} exchange, as well as Ca^{2+} and dinitrophenol-stimulated ATPase, *in vitro*, while ethanol had no significant effect [6]. These investigators suggested that acetaldehyde interacts with mitochondrial membranes. To the authors' knowledge, the effects of acetaldehyde on $Na^+ + K^+$-ATPase have not been investigated.

It is of interest that the aldehydes, 3,4-dihydroxyphenylglycolaldehyde, 3,4-dihydroxyphenylacetaldehyde and 5-hydroxyindoleacetaldehyde, derived from the corresponding biogenic amines by monoamine oxidase, were reported to bind "irreversibly" to membrane fractions of brain homogenates [7–9]. In addition, it has been demonstrated that phenyl-substituted aldehydes inhibit oxidative enzymes in mitochondrial fractions [10, 11]. Since it is possible that the biogenic aldehydes may alter the function of cell membranes, and inasmuch as $Na^+ + K^+$-ATPase is a membrane-bound enzyme involved in the active transport of Na^+, it is of interest to study the effects of biogenic aldehydes, acetaldehyde and other aldehydes on brain $Na^+ + K^+$-ATPase.

MATERIALS AND METHODS

Tris-base (Tris [hydroxymethyl] aminomethane), Tris–ATP, histidine-HCl, EDTA, sodium deoxycholate, L-norepinephrine-HCl, dopamine-HCl and indoleacetaldehyde (as the sodium bisulfite complex) were purchased from Sigma Chemical Co. Phenylacetaldehyde, benzaldehyde, acetaldehyde and propionaldehyde were purchased from Aldrich Chemical Co. Ethanol was U.S.P. pure ethyl alcohol from U.S. Industrial Chemicals. All other chemicals were of the highest quality available. Triple-distilled water was used to make the solutions.

Microsomal enzyme preparation. Adult DBA/6J/IBG mice of either sex, weighing 20–25 g, were decapitated, and the entire brain was rapidly removed, weighed and homogenized in 9 vol of homogenizing solution containing 0·25 M sucrose, 5 mM EDTA, 5 mM histidine and 0·05% deoxycholate, adjusted to pH 6·8 with Tris-base. The homogenate was initially centrifuged at 1000 *g* for 10 min to sediment the nuclei and cell debris. The resulting supernatant fluid was centrifuged at 10,000 *g* for 10 min to separate

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the crude mitochondrial fraction from the microsome-containing supernatant fluid. This supernatant fluid was centrifuged at 160,000 *g* for 60 min to sediment the microsomes. The microsomal pellet was resuspended in the original volume of homogenizing solution and centrifuged as above. This washing procedure was repeated with a solution which contained 0·25 M sucrose, 1 mM EDTA and 5 mM histidine (pH 7·0). Finally, the microsomal pellet was resuspended in a sufficient volume of the above solution to give about 5 mg protein/ml and was stored frozen until used. The enzyme in the frozen state was stable for at least 2 months. This enzyme preparation was found to possess greater activity than a preparation obtained without 0·05% deoxycholate in the medium. Since aldehydes could react with the lipid moiety of the membranes, the inhibitory effects on these preparations were determined. The degree of inhibition was the same with enzyme prepared in the presence or absence of deoxycholate.

Enzyme assay. The typical reaction mixture contained 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 100 mM Tris buffer (pH 7·4), 50 μg enzyme protein and 3 mM Tris–ATP, in a final volume of 1 ml. The Mg^{2+}-activated ATPase was determined in the absence of Na^+ and K^+. The mixture was preincubated for 5 min at 37°; then the reaction was started by the addition of ATP, and it continued for 5 min at 37°. The reaction was stopped by addition of 0·5 ml silicotungstic acid reagent, as described by Gibbs *et al.* [12]. Inorganic phosphate was determined by the method of Martin and Dotty [13]. The $Na^+ + K^+$-ATPase activity was calculated by subtraction of the activity of the Mg^{2+}-ATPase from the total activity. ATPase activity is expressed as umoles Pi liberated/mg of protein/hr. All assays were carried out in duplicate.

Activity of K^+ ion-dependent *p*-nitrophenylphosphatase (an acyl phosphate phosphohydrolase, EC 3.6.1.7) was determined spectrophotometrically, as recently described by Anderson [14]. Incubation mixtures were as described above except that 3 mM *p*-nitrophenylphosphate was the substrate, in place of Tris–ATP, with no Na^+ ion present. The extinction coefficient of *p*-nitrophenol at pH 7·4 was calculated from the Henderson–Hasselbach equation, $E_{405} = 1{\cdot}43 \times 10^4$. The p*K*a (7·04) and the molar extinction coefficient ($E_{405} = 1{\cdot}83 \times 10^4$ in 0·01 M NaOH) used in these calculations were reported by Kezdy and Bender [15]. The calculated extinction coefficient was verified experimentally. Tris-HCl, histidine-HCl, glycyl-glycine and aldehyde inhibitors at several concentrations were found to have no effect on the extinction coefficient. The rate of *p*-nitrophenol formation were determined at 405 nm with a Beckman 2400 recording spectrophotometer. The difference between activities in the presence or absence of K^+ ion was calculated and reported as the K^+-dependent *p*-nitrophenylphosphatase activity [16].

Protein was determined by the Biuret method or the method of Lowry *et al.* [17], with bovine serum albumin as the standard.

Preparation and assay of aldehydes. 3,4-Dihydroxyphenylglycolaldehyde and 3,4-dihydroxyphenylacetaldehyde were obtained from L-norepinephrine-HCl and dopamine-HCl by utilizing a partially purified monomaine oxidase, as previously described [18, 19]. Indoleacetaldehyde was prepared free of sodium bisulfite, as described by Erwin *et al.* [20]. The concentrations of all aldehydes were determined by the method described by Deitrich *et al.* [21] or by the chemical assay described by Sawicki *et al.* [22].

Determination of reversibility. For the dilaysis experiment, a mixture of enzyme, aldehyde and 0·1 M Tris buffer (pH 7·4) was incubated for 10 min at 37°, then dialyzed against a 1000-fold excess of solution containing 0·1 M Tris buffer (pH 7·4) and 1·0 mM EDTA for 12–24 hr at 2–4°. Enzyme in Tris buffer without aldehyde was similarly dialyzed in a separate container. ATPase activity and protein concentration were determined on each dialyzed fraction.

Sephadex G-50 was allowed to hydrate in 0·1 M Tris buffer (pH 7·4) for 72 hr at 2–4°; then a 2·5 cm × 20 cm column was prepared and equilibrated with Tris buffer (pH 7·4) solution. A mixture of enzyme and aldehyde or enzyme without aldehyde, as described above, was passed through the column, and 1·5-ml fractions were collected. Aldehyde, protein and ATPase activities were determined on various fractions, and a complete separation of the enzyme and aldehyde was obtained.

RESULTS

The $Na^+ + K^+$-ATPase activity in mouse brain microsomal fractions ranged from 30 to 40 μmoles Pi formed/mg of protein/hr, and the ratio of $Na^+ + K^+$-ATPase to Mg^{2+}-ATPase activity ranged from 1·5 to 3·0. K^+ ion-dependent *p*-nitrophenylphosphatase activity was approximately 11 μmoles *p*-nitrophenol formed/hr/mg of protein. The enzyme activities were linear with protein concentrations (20–150 μg) in the reaction mixture; rates of Pi or *p*-nitrophenol formation were linear for 5 min, after which a gradual decrease in the rates was observed.

As shown in Table 1, various aldehydes produced

Table 1. Comparison of inhibition of ATPase by aldehydes and ethanol

Inhibitor	Conc (moles/l.)	% Inhibition $Na^+ + K^+$-ATPase	% Inhibition Mg^{2+}-ATPase
Acetaldehyde	2×10^{-2}	41·5	3·4
Acetaldehyde	2×10^{-1}	97·7	46·7
Propionaldehyde	2×10^{-2}	64·5	28·3
Propionaldehyde	2×10^{-1}	100·0	46·0
Benzaldehyde	1×10^{-4}	2·1	10·9
Benzaldehyde	5×10^{-4}	44·7	8·2
Benzaldehyde	5×10^{-3}	100·0	59·1
Ethanol	2×10^{-2}	0	2·8
Ethanol	2×10^{-1}	10·4	0
Ethanol (1 mM K^+)	2×10^{-2}	0	9·5
Ethanol (1 mM K^+)	2×10^{-1}	4·2	2·7

* The reaction mixture contained 100 mM NaCl, 20 mM KCl, 5 mM $MgCl_2$, 100 mM Tris buffer (pH 7·4), 50 μg enzyme protein and 3 mM Tris-ATP, in a final volume of 1 ml. The reaction was initiated by addition of Tris-ATP after a 5-min incubation at 37°. After 5 min, the reaction was stopped and the μmoles of Pi formed/hr/mg of protein was determined in the presence of various inhibitors at the concentrations indicated. $Na^+ + K^+$-ATPase and Mg^{2+}-ATPase activities were determined as described in the text; the activities (without inhibitor) ranged from 14·05 to 32·99 and 11·9 to 18·7 μmoles Pi formed/hr/mg of protein respectively.

Table 2. Comparison of inhibition of *p*-nitrophenylphosphatase by aldehydes and ethanol*

Inhibitor	Conc (moles/l.)	% Inhibition K^+-pNPPase†	% Inhibition Mg^{2+}-pNPPase†
Acetaldehyde	2×10^{-2}	2·7	0
Acetaldehyde	2×10^{-1}	94·4	41·9
Propionaldehyde	2×10^{-2}	61·7	0
Propionaldehyde	2×10^{-1}	91·2	43·4
Benzaldehyde	1×10^{-4}	0	0
Benzaldehyde	5×10^{-3}	29·6	8·5
Benzaldehyde	$2{\cdot}5 \times 10^{-2}$	66·0	18·6
Ethanol (20 mM KCl)	2×10^{-1}	0	0
Ethanol (1 mM KCl)	2×10^{-1}	7·9	0

* The reaction mixture contained 20 mM KCl, 5 mM $MgCl_2$, 100 mM Tris buffer (pH 7·4), 5 μg enzyme protein and 3 mM *p*-nitrophenylphosphate, with an absence of Na^+ ion. After the mixture was incubated in 1·0 ml (final volume) for 5 min at 37°, the reaction was initiated by addition of *p*-nitrophenylphosphate. Rate of *p*-nitrophenol formation was determined spectrophotometrically at 405 nm as described in the text. K^+-stimulated phosphatase activity was taken as the difference between rates obtained in the absence or presence of 20 mM KCl. K^+-stimulated *p*-nitrophenylphosphatase activity, in the absence of inhibitor, ranged from 9·5 to 12·7 μmoles *p*-nitrophenol formed/hr/mg of protein.

† pNPPase refers to *p*-nitrophenylphosphatase. Each value represents the average of three separate enzyme activity determinations.

a marked inhibition of $Na^+ + K^+$-ATPase activity, and the extent of inhibition was dependent upon the aldehyde employed. Virtually complete inhibition of the enzyme activity was obtained with 0·2 M acetaldehyde or propionaldehyde, whereas the concentration of benzaldehyde required to inhibit $Na^+ + K^+$-ATPase activity by 100 per cent was 5·0 mM (Table 1). $Na^+ + K^+$-ATPase was more sensitive to inhibition by aldehydes than was Mg^{2+}-ATPase, and concentrations of aldehydes producing virtually complete inhibition of $Na^+ + K^+$-ATPase inhibited Mg^{2+}-ATPase by only 50–60 per cent. Of particular interest is the fact that ethanol, at concentrations as high as 0·2 M, caused only a slight (*ca.* 10 per cent) inhibition of the microsomal $Na^+ + K^+$-ATPase from mouse brain. Since it has been reported that ethanol inhibition of $Na^+ + K^+$-ATPase from rat brain microsomes is competitive with K^+ ion [2], the effects of decreasing the K^+ ion concentration from 20 to 1 mM in the reaction mixture were determined. The degree of inhibition of mouse brain microsomal $Na^+ + K^+$-ATPase by ethanol was not appreciably altered by this procedure (Table 1).

The terminal reaction catalyzed by $Na^+ + K^+$-ATPase is generally considered to be a K^+-dependent dephosphorylation. The K^+-dependent *p*-nitrophenyl phosphatase activity which appears in ATPase preparations has been shown to be similar in many respects to this second reaction [23]. Therefore, it was of interest to determine the effects of various aldehydes on this reaction. The results in Table 2 show that aldehydes inhibited K^+ ion-dependent *p*-nitrophenylphosphatase activity; however, the enzyme activity was 10- to 20-fold less sensitive to inhibition than was $Na^+ + K^+$-ATPase.

Inasmuch as it has been reported that aldehydes become "irreversibly" bound to cell membrane fractions [24], and in order to more fully study the nature of inhibition of $Na^+ + K^+$-ATPase by aldehydes, the reversibility of inhibition was determined. As shown in Table 3, inhibition of $Na^+ + K^+$-ATPase and *p*-nitrophenylphosphatase activities by benzaldehyde was completely reversible upon separation of the enzyme from 10^{-2} M to 10^{-3} M aldehyde by Sephadex G-50 chromatography. The inhibition did not appear to be readily reversible by dialysis in a cellulose bag. When the enzyme fraction and aldehyde incubation mixtures were placed in a cellulose dialysis bag and dialyzed against 1000-fold excess of Tris buffer (pH 7·4) for 24 hr, it was shown, using benzaldehyde-7C-^{3}H, that aldehyde was removed from the bag. However, the enzyme was unstable under these conditions, and incubation mixtures (with or without aldehyde) lost a high percentage of their activity (*ca.*

Table 3. Reversibility of $Na^+ + K^+$-ATPase and K^+-stimulated *p*-nitrophenylphosphatase inhibition by Benzaldehyde*

Experimental condition†	Benzaldehyde conc (moles/l.)	Enzyme activity‡	Inhibitoon (%)	Reversal of inhibition (%)
pNPPase before	0	11·1		
pNPPase before	10^{-2}	4·4	60·4	
pNPPase after	0	11·2	0	
pNPPase after	10^{-2}	11·0	1·0	98·4
ATPase before	0	32·5		
ATPase before	4×10^{-3}	0	100	
ATPase after	0	30·62	0	
ATPase after	4×10^{-3}	31·00	0	100

* These studies were performed as described in the text. Solutions containing enzyme protein and either 10^{-3} M or 10^{-2} M benzaldehyde in 0·1 M Tris buffer (pH 7·4) were incubated at 37° for 10 min and subsequently passed through a Sephadex G-50 column. Fractions were collected as described in the text and those containing protein were assayed for $Na^+ + K^+$-ATPase of K^+-stimulated *p*-nitrophenylphosphatase.

† pNPPase represents *p*-nitrophenylphosphatase and ATPase represents $Na^+ + K^+$-ATPase, before or after dialysis through a Sephadex G-50 column.

‡ pNPPase activity refers to μmoles *p*-nitrophenol formed/hr/mg of protein; ATPase activity is μmoles Pi formed/hr/mg of protein.

Table 4. Effect of cysteine on inhibition of ATPase by benzaldehyde*

Experimental condition	Enzyme activity†		% Inhibition	
	$Na^+ + K^+$-ATPase	Mg^{2+}-ATPase	$Na^+ + K^+$-ATPase	Mg^{2+}-ATPase
Complete reaction mixture (CRM)	37·7	12·4		
CRM + cysteine (10^{-2} M)	35·5	12·6	5·9	0
CRM + benzaldehyde (8×10^{-4} M)	14·3	7·8	62·1	37·1
CRM + cysteine (10^{-2} M) and benzaldehyde (8×10^{-4} M)	32·2	11·4	14·6	8·1

* Experiments were performed as described in the text and Table 1, except that various concentrations of cysteine (as indicated) were added to the incubation mixture before benzaldehyde.

† Enzyme activity is expressed as μmoles Pi formed/hr/mg of protein. Each value represents the average of three separate experiments.

80 per cent) during the dialysis procedure. In this instance, passing the enzyme through a Sephadex G-50 column was a superior method for determining reversibility.

Table 5. Effects of cysteine and 2-mercaptoethanol on inhibition of *p*-nitrophenylphosphatase by benzaldehyde*

Experimental condition	Enzyme activity†	% Inhibition
Complete reaction mixture (CRM)	12·7	
CRM + cysteine	10·1	20·5
CRM + mercaptoethanol	12·9	0
CRM + benzaldehyde	8·5	33·1
CRM + cysteine and Benzaldehyde	9·8	22·8
CRM + mercaptoethanol and Benzaldehyde	11·3	11·0

* Experiments were performed as described in the text and Table 2. Sufficient cysteine or mercaptoethanol was added to give final concentrations of 10^{-2}M in the reaction mixtures before benzaldehyde (3×10^{-3} M).

† Enzyme activity is expressed as μmoles *p*-nitrophenol formed/hr/mg of protein. Each value represents the average of four experiments.

The data presented in Tables 4 and 5 show that cysteine or 2-mercaptoethanol in the reaction mixture protected $Na^+ + K^+$-ATPase or *p*-nitrophenylphosphatase from inhibition by benzaldehyde. Relatively high concentrations of sulfydryl compounds were required to achieve complete protection.

As shown in Tables 6 and 7, the biogenic aldehydes were more potent inhibitors of $Na^+ + K^+$-ATPase or *p*-nitrophenylphosphatase activity than were short-chain aliphatic aldehydes or benzaldehyde. The concentrations of 3,4-dihydroxyphenylglycoaldehyde or 3,4-dihydroxyphenylacetaldehyde required to produce approximately 50 per cent inhibition of $Na^+ + K^+$-ATPase were 1×10^{-5} M and 3×10^{-5} M respectively. Mg^{2+} and *p*-nitrophenylphosphatase were less sensitive than $Na^+ + K^+$-ATPase to inhibition by the biogenic aldehydes. Indoleacetaldehyde did not inhibit $Na^+ + K^+$-ATPase, *p*-nitrophenylphosphatase of Mg^{2+}-ATPase at concentrations up to $1·7 \times 10^{-4}$ M. It is of interest that $Na^+ + K^+$-ATPase activity, as well as *p*-nitrophenylphosphatase activity, was increased 20–30 per cent by this aldehyde. Because of the low solubility, higher concentrations of the aldehyde were not employed. Phenylacetaldehyde was a relatively potent inhibitor of $Na^+ + K^+$-ATPase; concentrations of $6·3 \times 10^{-5}$ M produced approximately 33 per cent inhibition. However, this

Table 6. Inhibition of brain microsomal $Na^+ + K^+$-ATPase by biogenic aldehydes*

Aldehyde†	Conc (moles/l.)	Enzyme activity‡		% Inhibition	
		$Na^+ + K^+$-ATPase	Mg^{2+}-ATPase	$Na^+ + K^+$-ATPase	Mg^{2+}-ATPase
None	0	30·9	18·3		
DHPGAl	$6·8 \times 10^{-6}$	19·1	16·4	38·2	10·4
DHPGAl	$1·4 \times 10^{-5}$	14·4	14·4	53·4	21·3
DHPGAl	$2·4 \times 10^{-4}$	4·4	17·3	85·8	5·5
DHPAAl	$1·3 \times 10^{-5}$	16·8	17·6	45·7	4·0
DHPAAl	$3·6 \times 10^{-5}$	12·5	18·2	59·5	0
PAAl	$1·6 \times 10^{-5}$	24·7	18·3	16·0	0
PAAl	$6·3 \times 10^{-5}$	20·8	16·5	32·6	9·9
IAAl	$7·0 \times 10^{-5}$	37·9	18·7	(22·6)§	0
IAAl	$1·7 \times 10^{-4}$	40·1	18·0	(29·8)§	0

* Enzyme assay procedures were as described in the text and Table 1. Various biogenic aldehydes, in the final concentrations indicated, were incubated with the enzyme for 5 min prior to initiating the reaction by addition of ATP.

† DHPGAl, 3,4-dihydroxyphenylglycolaldehyde; DHPAAl, 3,4-dihydroxyphenylacetaldehyde; PAAl phenylacetaldehyde; and IAAl, indoleacetaldehyde.

‡ Enzyme activity is expressed as μmoles Pi formed/hr/mg of protein. Each value represents the average of three or four separate experiments.

§ Represents an increase in $Na^+ + K^+$-ATPase activity rather than inhibition.

Table 7. Inhibition of brain microsomal *p*-nitrophenylphosphatase by biogenic aldehydes*

Aldehyde†	Conc (moles/l.)	Enzyme activity‡	% Inhibition
None	0	11·2	
DHPGAl	3×10^{-6}	10·3	8·0
DHPGAl	$1{\cdot}4 \times 10^{-5}$	7·6	32·1
DHPAAl	$3{\cdot}8 \times 10^{-5}$	9·4	16·1
DHPAAl	$1{\cdot}3 \times 10^{-4}$	6·3	43·8
PAAl	$1{\cdot}6 \times 10^{-5}$	11·1	0
PAAl	$6{\cdot}3 \times 10^{-5}$	11·0	0
IAAl	7×10^{-5}	14·3	(27·7)§
IAAl	$1{\cdot}7 \times 10^{-4}$	17·5	(56·3)§

* Enzyme assay procedures were as described in the text and Tables 2 and 6.

† DHPGAl, 3,4-dihydroxyphenylglycolaldehyde; DHPAAl, 3,4-dihydroxyphenylacetaldehyde; PAAl, phenylacetaldehyde; and IAAl, indoleacetaldehyde.

‡ Enzyme activity is expressed as μmoles *p*-nitrophenol formed/hr/mg of protein. Each value represents the average of three separate experiments.

§ Represents an increase in *p*-nitrophenylphosphatase activity rather than inhibition.

aldehyde had little effect on K^+-activated *p*-nitrophenylphosphatase.

Since it has been reported [2, 3] that inhibition of $Na^+ + K^+$-ATPase by ethanol is competitive with K^+, it was of interest to determine the effects of varying the concentration of Na^+ or K^+ in the reaction mixture upon inhibition of the enzyme by aldehydes. The degree of inhibition by benzaldehyde (Table 8) with concentrations of Na^+ ranging from 10 to 200 mM (with a constant 20 mM concentration of K^+) varied by only 19 per cent. In addition, varying the K^+ concentration from 1 to 40 mM, with a constant concentration of Na^+ (100 mM), did not markedly affect the inhibition of $Na^+ + K^+$-ATPase activity. Indeed, the data presented in Fig. 1 show that inhibition was non-competitive with K^+ ion as the variable activator.

The rates of $Na^+ + K^+$-ATPase activity were determined at various concentrations of ATP in the presence or absence of benzaldehyde in the reaction mixture. As shown in Fig. 2, the inhibition was non-competitive by the Lineweaver–Burk plot, with a K_i value for benzaldehyde of $3{\cdot}65 \times 10^{-4}$ M.

Table 8. Effects of varying Na^+ ion concentration on inhibition of $Na^+ + K^+$-ATPase by Benzaldehyde*

Na^+ (mM)	Benzaldehyde	Enzyme activity†	% Inhibition
10	Absent	11·5	
10	Present	4·9	57·4
100	Absent	31·8	
100	Present	17·8	44·0
200	Absent	17·1	
200	Present	10·5	38·6

* Enzymes were assayed, as described in the text and Table 1, in the absence or presence of 4×10^{-4} M benzaldehyde and at various concentrations of Na^+ ion, as indicated.

† Enzyme activity is expressed as μmoles Pi formed/hr/mg of protein. Each value represents the average of at least three experiments.

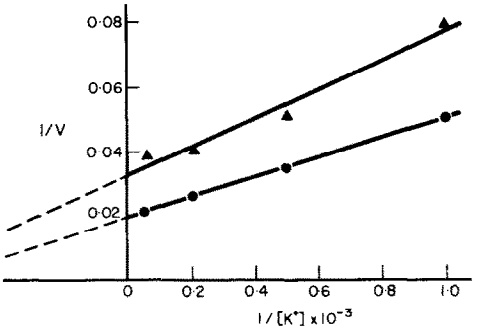

Fig. 1. Kinetics of brain microsomal $Na^+ + K^+$-ATPase inhibition by benzaldehyde. The reaction mixtures and assay conditions were as described in Table 1, except that the K^+ ion concentration was varied as indicated. Key: (●) represents control without inhibitor; (▲) with 4×10^{-4} M benzaldehyde. The K_m value for K^+ ion was $1{\cdot}6 \times 10^{-3}$ M, and the K_i value for benzaldehyde was 5×10^{-4} M.

DISCUSSION

Previous investigators have reported [2, 3] that 0·22 M ethanol, in reaction mixtures with a low K^+ concentration (1 mM), produced about 50 per cent inhibition of microsomal $Na^+ + K^+$-ATPase from the rat and guinea pig cerebral cortex. However, in the present studies, in which microsomal fractions from mouse brain were used, ethanol (at 0·22 M final concentration) did not significantly alter the enzyme activity at K^+ concentrations of 1–20 mM. It is possible that brain $Na^+ + K^+$-ATPase from various species, and even various strains within a species, differs in sensitivity to inhibition by ethanol.

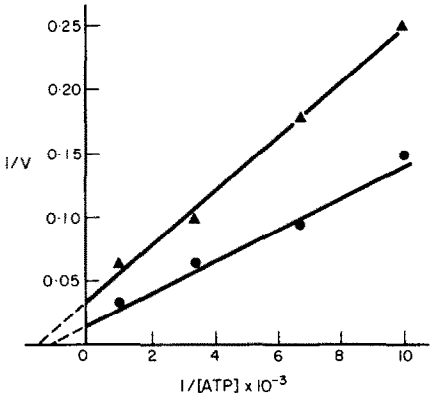

Fig. 2. Kinetics of mouse brain microsomal $Na^+ + K^+$-ATPase inhibition by benzaldehyde. The reaction mixtures and assay conditions were as described in Table 1, except that concentration of Tris-ATP was varied as indicated. Each point represents the average of four experiments performed in duplicate. ATPase activity is expressed as μmoles Pi formed/hr/mg of protein. Key: (●) represents control without inhibitor; (▲) with 4×10^{-4} M benzaldehyde. The K_m value for ATP was $7{\cdot}6 \times 10^{-4}$ M, and the K_i value for benzaldehyde was $3{\cdot}6 \times 10^{-4}$ M.

The effects of ethanol, as well as of various aldehydes, on K^+-stimulated *p*-nitrophenylphosphatase were also investigated. This enzyme activity, associated with the $Na^+ + K^+$-ATPase, is believed to be a model for the K^+-dependent dephosphorylation step in the overall reaction [23]. The present studies revealed that this enzyme activity from mouse brain microsomes was not significantly inhibited by 0·1 M ethanol.

It is of interest that acetaldehyde, the initial metabolic product of ethanol, was at least 20 times more potent than ethanol in producing inhibition of $Na^+ + K^+$-ATPase activity of mouse brain microsomes. The effects of acetaldehyde and other aliphatic aldehydes, as well as benzaldehyde, on enzyme systems have been reported [10, 11]. Recently, Blass and Lewis [25] observed that mM concentrations of acetaldehyde inhibited pyruvate dehydrogenase activity in ox brain and kidney. The inhibition was reversible by dilution and partially prevented by equimolar concentrations of cysteine. Similarly, the present study showed that aldehyde inhibition of $Na^+ + K^+$-ATPase was reversible by dialysis on a Sephadex G-50 column and that inhibition was prevented by this amino acid.

These observations are consistent with those of Ungar *et al.* [24] which showed that cysteine prevented the binding of biogenic aldehydes to rat brain membranes. It is well known that cysteine forms thiazolidine derivatives with aldehydes [26] and that the formation of such complexes causes a decrease in the concentration of 'free' aldehydes in the reaction mixture. The present data indicate that the reactivity of the aldehyde and the nature of the substituent on the aldehydic group are involved in the inhibition of $Na^+ + K^+$-ATPase. For example, benzaldehyde produced more inhibition of the enzyme activity than did acetaldehyde, and phenylacetaldehyde was slightly more potent than benzaldehyde. Addition of catecholhydroxyl groups markedly increased the inhibitory effects of the aldehydes. The catechol moiety may be involved in binding of the aldehydes to the enzyme and, since indoleacetaldehyde did not inhibit enzyme activity, substitution of the indole group is apparently detrimental to binding.

Aldehydes produced a non-competitive inhibition of $Na^+ + K^+$-ATPase with ATP as the variable substrate, and, as shown in Table 8 and Fig. 1, these compounds caused a non-competitive inhibition with either Na^+ or K^+ as the variable ion. The non-competitive nature of the inhibition indicates that the aldehydes inhibit $Na^+ + K^+$-ATPase by a different mechanism than does ethanol or oubain, which produced a competitive-type inhibition with K^+ ion and which are believed to inhibit the dephosphorylation step. Indeed, if a common phosphorylated enzyme intermediate were formed from phosphorylation by either ATP or *p*-nitrophenylphosphatase, and if dephosphorylation were rate limiting, one would expect equivalent inhibition of the phosphatase and ATPase reactions by compounds acting on this step. Since $Na^+ + K^+$-ATPase was 10–20 times more sensitive than the phosphatase to inhibition by aldehydes, it might be concluded that these inhibitors act on a step other than dephosphorylation in the complex reaction catalyzed by $Na^+ + K^+$-ATPase. Studies are presently being conducted to elucidate more fully the mechanism of inhibition of this enzyme by aldehydes.

Israel and Kuriyama [27] found that chronic ethanol administration had no effect on synaptosomal and microsomal $Na^+ + K^+$-ATPase activity in mouse brain. Since the inhibition by ethanol or aldehydes is reversible, alterations of $Na^+ + K^+$-ATPase activity in isolated and 'washed' membrane fractions might not be observed unless these substances induced changes in membrane or protein synthesis. Certainly, the physiological and pharmacological significance of $Na^+ + K^+$-ATPase inhibition by these compounds remains to be clarified. It is of interest that the concentrations of acetaldehyde in blood and brain after ethanol administration have been found to range from 10^{-4} M to 10^{-5} M [28]; and, according to the observations of Erwin and Deitrich [29], these concentrations are greater than the K_m values for acetaldehyde with brain aldehyde dehydrogenase. It is possible that, after ethanol administration, 'saturation' of brain aldehyde dehydrogenase with acetaldehyde might cause an increase in 3,4-dihydroxyphenylacetaldehyde, *in vivo*. The latter aldehyde produced marked inhibition of $Na^+ + K^+$-ATPase at 10^{-5} M concentrations.

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