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IN VIVO AND *IN VITRO* EFFECTS OF ALDRIN ON RAT BRAIN SYNAPTOSOMAL Mg^{2+} AND Na^+,K^+-ADENOSINE TRIPHOSPHATASE

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Abstract—Aldrin, a chlorinated hydrocarbon, inhibited rat brain synaptosomal membrane-bound Na^+,K^+-adenosine triphosphatase (ATPase) and Mg^{2+}-ATPase activities under *in vivo* and *in vitro* conditions. Na^+,K^+-ATPase was non-competitively inhibited whereas Mg^{2+}-ATPase was inhibited uncompetitively. Arrhenius plots of both these ATPases without aldrin under *in vivo* and *in vitro* conditions were found to be linear. In the presence of aldrin, on the other hand, Arrhenius plots of the same ATPases were nonlinear. Slopes of Arrhenius plots of both ATPases under *in vivo* and *in vitro* condition were changed with change in temperature with aldrin. The activation energy (AE) of Na^+,K^+-ATPase and Mg^{2+}-ATPase activities were changed over the temperature range 15–40° in the presence of aldrin. These results thus suggest that aldrin increases the lipid fluidity of the synaptosomal membrane which may be a cause of inhibition of neuronal membrane-bound Na^+,K^+ and Mg^{2+}-ATPase activities.

Aldrin (hexahydromethanonaphthalene), a chlorinated hydrocarbon is a well-known insecticide. It is well known that organochlorine insecticides are highly lipophilic in nature and accumulates in the tissue lipid [1]. These classes of insecticides generally affect the reproductive function [2, 3], cellular function [4] and hepatobiliary function [5, 6] and also produce hepatic carcinoma [7, 8]. Several workers have found that DDT and other chlorinated hydrocarbon insecticides inhibit the Na^+,K^+-adenosine triphosphatase (ATPase) and Mg^{2+}-ATPase activities of nerve, muscle and other parts of the animal system [9–12]. Na^+,K^+ activated Mg^{2+}-dependent ATPases (Na^+,K^+-ATPase; EC 3.6.1.3) one of the most important intrinsic membrane-bound enzymes [13, 14], is associated with the propagation of nerve impulse [15, 16]. This enzyme is known to be involved in the regulation of catecholaminergic and serotonergic activity [17–21]. Recently, we have also observed that aldrin alters the catecholaminergic and serotonergic activity in mammalian brain [22, 23]. Though several studies [11, 14, 15, 24] of the effect of chlorinated hydrocarbon on the membrane-bound ATPases have been carried out, the behaviour of these membrane-bound enzymes in relation to the effect of temperature appear to be complex [25]. In the present investigation the effect of aldrin under *in vivo* and *in vitro* conditions on Mg^{2+}-ATPase and Na^+,K^+-ATPase of rat brain synaptosome in relation to the changes in membrane lipid fluidity characterized by Arrhenius parameters have been studied.

MATERIALS AND METHODS

Male albino rats (Charles Foster strain), aged 60 days (70–90 g wt), maintained on a standard laboratory diet and water *ad libitum* were used in the present study. Aldrin was obtained as a gift from NOCIL (National Organic Chemical Industries Ltd, Calcutta Branch, India). ATP (adenosine triphosphate, grade II) was purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Other experimental reagents were of analytical grade.

In vivo *treatment with aldrin.* Rats were divided into three separate groups. Each group contained 3–5 rats. First and second groups were treated (p.o.) with aldrin at 0.4 mg/kg and 4.0 mg/kg respectively. Animals of the third group treated with the same amount of vehicle corresponding to the experimental group, first or second, through the same route, was considered as control. After 2 hr of vehicle or aldrin administration, the animals of both control and experimental groups were killed by cervical dislocation between 10.00–11.00 a.m. unless otherwise mentioned.

Preparation of synaptosomes. Immediately after death the brains were quickly removed and placed in 10 vol. of 0.25 M sucrose solution. Synaptosomes of each brain were prepared by a sucrose density gradient procedure according to the method of Gray and Whittaker [26] and modified by Bradford *et al.* [27]. This synaptosomal preparation remained intact during *in vivo* and *in vitro* treatments with aldrin and even during enzyme assays (after both *in vivo* and *in vitro* treatments). This was confirmed by the absence of occluded lactate dehydrogenase activity (Table 1) in synaptosomal preparation under those conditions. The appreciable activity of the enzyme was observed after sonication of the synaptosomal preparation in the absence and presence of aldrin (Table 1).

In vitro *treatment with aldrin.* Brains of normal adult male albino rats were used for synaptosomal preparation following the procedure as described

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Table 1. Effect of *in vivo* and *in vitro* aldrin treatments and sonication on synaptosomal occluded lactic dehydrogenase (LDH) activity (μmol of NADH oxidized/min) of rat brain

	LDH activity (μmol of NADH oxidized/min)					
	*in vivo**		*in vitro*§		Sonication**	
Treatment	Before assay†	During assay‡	Before assay‖	During assay¶	Before sonication	After sonication
Control	—	—	—	—	—	3.44 ± 0.069
Aldrin	—	—	—	—	—	3.52 ± 0.280

Values were expressed as means ± SE of three separate determinations.
* Synaptosome was prepared from control and aldrin (4.0 mg/kg, for 2 hr)-treated rat.
† LDH was estimated before Na^+,K^+ and Mg^{2+}-ATPase assay.
‡ LDH was estimated by taking an aliquot from assay medium of Na^+,K^+- and Mg^{2+}-ATPase after the incubation was over.
§ Synaptosome was prepared and treated with aldrin (150 nM) for 30 min at 37°.
‖ LDH was estimated before Na^+,K^+- and Mg^{2+}-ATPase assay.
¶ LDH was estimated by taking an aliquot from assay medium of Na^+,K^+- and Mg^{2+}-ATPase after the incubation was over.
** Synaptosome was prepared and sonication (50 Hz for 30 sec × 3) was carried out.

above. Synaptosomal protein was treated with aldrin [20 ng/mg protein (15 nM) or 200 ng/mg protein (150 nM)] at 37° for 30 min unless otherwise stated. Thirty-min preincubation showed a maximum inhibitory effect of aldrin.

Estimation of Na^+,K^+*-ATPase and* Mg^{2+}*-ATPase activities.* Na^+,K^+-ATPase and Mg^{2+}-ATPase activities were determined according to the method of Yamamoto *et al.* [28]. The enzyme activity for a Lineweaver–Burk plot was measured with varying substrate (ATP) concentration (0.5–6.0 mM). In Arrhenius plots 6 mM substrate concentration was used with varying temperatures (15–40°). Other details are as described above. Enzyme activity was expressed as nmol of inorganic phosphate liberated/mg protein/hr.

Estimation of lactic dehydrogenase (LDH) activity. LDH activity was determined according to the method of Kornberg [29].

Estimation of protein. The protein content of the enzyme preparation was determined according to the method of Lowry *et al.* [30].

Expression of results. Each point on the graphs is the mean ± SE of three different synaptosomal preparations and each preparation was assayed three times. Double-reciprocal plots of kinetic data were prepared according to the method of Lineweaver and Burk [31]. Data were subjected to regressional analysis and the regression lines plotted for best straight-line fit.

Statistical analysis. The statistical significance of difference between means of control and experimental values was determined by two-tailed Student's *t*-test.

RESULTS

Table 2 describes the *in vivo* effect of aldrin (0.4 mg/kg or 4.0 mg/kg, p.o.) following 1–4 hr of its administration on the whole brain synaptosomal Na^+,K^+- and Mg^{2+}-ATPase activities. It is clear from Table 2 that aldrin at lower dose (0.4 mg/kg) inhibited (36 to 37%) synaptosomal Mg^{2+}-ATPase without any significant change of Na^+,K^+-ATPase activity. Higher dose (4.0 mg/kg) of aldrin inhibited both Na^+,K^+-ATPase and Mg^{2+}-ATPase. The inhibition of Na^+,K^+-ATPase was maximum (48.63%) after 2 hr of aldrin treatment and then gradually reduced with time. Maximum inhibition (55.18%) of Mg^{2+}-ATPase was also observed at 2 hr and the percentage inhibition remained the same even with increase of time after aldrin administration.

Table 3 describes the *in vitro* effect (10–60 min) of aldrin (15–150 nM) on synaptosomal Na^+,K^+- and Mg^{2+}-ATPase activities. It is evident from Table 3 that aldrin at lower concentrations (15 nM) inhibited Mg^{2+}-ATPase only whereas at higher concentrations (150 nM) both Na^+,K^+- and Mg^{2+}-ATPase were inhibited. The inhibition of Mg^{2+}-ATPase was significant after 20–60 min incubation with aldrin and the maximum inhibition (33.69% and 52.15% for 15 nM and 150 nM respectively) was observed after 30-min incubation with aldrin and persisted up to 1 hr. The Na^+,K^+-ATPase was significantly inhibited after 10–45-min incubation with a higher concentration of aldrin. The maximum inhibition (45.93%) was observed after 30-min incubation and then gradually withdrawn.

Results presented in Table 4 showed that removal of aldrin by 3-hr dialysis reversed its (aldrin) inhibitory effect on Na^+,K^+-ATPase activity of synaptosomal membrane whereas aldrin-induced inhibition of synaptosomal membrane-bound Mg^{2+}-ATPase activity was not reversed following 3 hr or more dialysis.

It is evident from Table 5 that aldrin both at low and high doses under *in vivo* conditions significantly lowered both K_m (27.25% to 36.96%) and V_{max} (41.70 to 61.13%) of Mg^{2+}-ATPase; whereas, only high doses of aldrin lowered the V_{max} value (47.05%) of Na^+,K^+-ATPase without affecting its K_m.

It appears from Table 6 that kinetic parameters (K_m and V_{max}) of Na^+-K^+-ATPase was not changed at low concentrations of aldrin. High concentrations of aldrin, on the other hand, showed a significant

Table 2. *In vivo* effect of aldrin (following 1–4 hr of its administration) on the activities of rat brain synaptosomal Na^+,K^+-ATPase and Mg^{2+}-ATPase

Treatment	Doses (mg/kg, p.o.)	ATPase activity (nmol Pi liberated/hr/mg protein)							
		Na^+,K^+-				Mg^{2+}			
		1 hr	2 hr	3 hr	4 hr	1 hr	2 hr	3 hr	4 hr
Control	—	354.50 ± 4.76	350.48 ± 5.61	348.00 ± 3.05	342.00 ± 8.08	321.50 ± 8.17	319.00 ± 12.85	320.40 ± 10.58	311.50 ± 5.96
Aldrin	0.4	330.00 ± 18.58	324.51 ± 14.60	336.00 ± 10.21	361.18 ± 21.73	205.21 ± 8.75*	200.00 ± 15.27†	200.98 ± 6.67*	198.00 ± 3.05*
	4.0	225.66 ± 6.98*	180.00 ± 5.50*	200.55 ± 9.27*	213.28 ± 5.29*	148.50 ± 5.96*	142.95 ± 6.48*	143.32 ± 1.34*	142.00 ± 4.93*

All values were expressed as means ± SE of three separate determinations.
Significantly different from control * $P < 0.001$; † $P < 0.005$.
Rats were treated with aldrin (0.4 or 4.0 mg/kg, p.o.).
Control rats were treated with vehicle and the animals were killed after 1–4 hr of aldrin or its vehicle administration.

Table 3. *In vitro* effect of aldrin (with varying preincubation time) on the activities of normal brain synaptosomal Na^+,K^+-ATPase and Mg^{2+}-ATPase at 37°

Treatment	Concentration (nM)	ATPase activity (nmol Pi liberated/hr/mg protein)									
		Na^+,K^+-					Mg^{2+}				
		10 min	20 min	30 min	45 min	60 min	10 min	20 min	30 min	45 min	60 min
Control	—	351.00 ± 8.38	350.52 ± 7.66	350.40 ± 5.61	349.00 ± 8.18	348.20 ± 5.54	319.63 ± 5.87	320.20 ± 5.30	319.20 ± 3.96	319.50 ± 5.00	320.20 ± 4.44
Aldrin	15.0	326.21 ± 7.63	330.22 ± 8.73	325.61 ± 20.33	331.00 ± 7.09	331.50 ± 8.97	319.23 ± 4.17	230.00 ± 8.66*	211.66 ± 6.00*	216.60 ± 4.74*	216.80 ± 4.77*
	150.0	308.67 ± 1.54	221.81 ± 8.38*	189.45 ± 10.00*	294.00 ± 2.88*	340.15 ± 1.06	318.23 ± 2.28	163.54 ± 5.50*	152.72 ± 12.47*	155.55 ± 7.09*	154.59 ± 6.51*

All values were expressed as means ± SE of three separate determinations.
Significantly different from control * $P < 0.001$.

Table 4. Reversibility test of synaptosomal Na^+,K^+-ATPase and Mg^{2+}-ATPase by aldrin

System	ATPase activity (nmol Pi liberated/hr/mg protein)					
	Na^+,K^+-			Mg^{2+}-		
	Before dialysis	After dialysis for 2 hr	After dialysis for 3 hr	Before dialysis	After dialysis for 2 hr	After dialysis for 3 hr
Control	345.61 ± 1.37	354.30 ± 3.63	341.24 ± 5.05	314.54 ± 3.46	318.42 ± 0.64	306.45 ± 8.42
Aldrin (150 nM)	177.58 ± 7.16*	260.98 ± 7.80*	329.00 ± 8.08	152.00 ± 4.61*	145.18 ± 0.15*	143.17 ± 11.80*

Each value was expressed as means ± SE of three separate determinations.
Enzyme was preincubated with or without aldrin for 30 min at 37° and dialysed against 0.1 M Tris–HCl buffer, pH 7.4 for 2 and 3 hr at 4°.
Significantly different from control * $P < 0.001$.

Table 5. *In vivo* effect of aldrin on K_m and V_{max} of synaptosomal Na^+,K^+-ATPase and Mg^{2+}-ATPase

Treatment	Doses (mg/kg, p.o.)	Na^+K^+-ATPase		Mg^{2+}-ATPase	
		K_m (mM)	V_{max} (nmol Pi/hr/mg protein)	K_m (mM)	V_{max} (nmol Pi/hr/mg protein)
Control	—	8.0 ± 1.15	755.55 ± 44.44	4.22 ± 0.20	571.75 ± 9.43
Aldrin	0.4	8.0 ± 1.15	755.55 ± 44.44	3.07 ± 0.14*	333.33 ± 38.49*
	4.0	8.0 ± 1.15	400.00 ± 57.73	2.66 ± 0.10*	222.22 ± 14.52*

All values are means ± SE of three separate determinations.
Significantly different from control * $P < 0.001$.
K_m and V_{max} were calculated from Lineweaver–Burk (LB) analysis.

Table 6. *In vitro* effect of aldrin on kinetic parameters (K_m and V_{max}) of synaptosomal Na^+,K^+-ATPase and Mg^{2+}-ATPase

Treatment	Concentration (nM)	Na^+,K^+-ATPase		Mg^{2+}-ATPase	
		K_m (mM)	V_{max} (nmol Pi/hr/mg protein)	K_m (mM)	V_{max} (nmol Pi/hr/mg protein)
Control	—	8.0 ± 1.01	755.55 ± 44.44	4.22 ± 0.20	571.95 ± 9.43
Aldrin	15.0	8.0 ± 1.01	755.55 ± 44.44	3.07 ± 0.15*	363.63 ± 19.39*
	150.0	8.0 ± 1.01	444.94 ± 64.06*	2.66 ± 0.095*	210.52 ± 6.44*

All values are means ± SE of three separate determinations.
Significantly different from control * $P < 0.001$.
K_m and V_{max} were calculated from Lineweaver–Burk (LB) analysis.

inhibition (41.11%) in V_{max} of Na^+,K^+-ATPase without changing its K_m value. Unlike kinetic parameters of Na^+,K^+-ATPase, the kinetic parameters (K_m and V_{max}) of Mg^{2+}-ATPase were significantly lowered both at low and high concentrations of aldrin.

Figure 1(a) and (b) represents Arrhenius plots of Na^+,K^+-ATPase and Mg^{2+}-ATPase, respectively, in synaptosomal preparation of brain of rat treated with low (0.4 mg/kg) and high (4.0 mg/kg) doses of aldrin. It is evident from Fig. 1(a) and (b) that Na^+,K^+-ATPase and Mg^{2+}-ATPase did not exhibit any transition temperature (TT) in control rat brain synaptosome. But treatment with aldrin both at low and high doses, exhibited TT in both Mg^{2+} and Na^+,K^+-ATPase. The values of Arrhenius plots analysis of Fig. 1(a) and (b) are represented in Table 5.

Figure 2(a) and (b) represents *in vitro* effects of aldrin (15 nM and 150 nM) on Arrhenius plots of rat brain synaptosomal Na^+,K^+-ATPase and Mg^{2+}-ATPase. Both Na^+,K^+-ATPase and Mg^{2+}-ATPase of brain synaptosome did not exhibit TT in the absence of aldrin (control); whereas, these two ATPases exhibited TT in the presence of both the concentrations of aldrin.

Table 7 represents Arrhenius plots of Na^+,K^+-ATPase and Mg^{2+}-ATPase of synaptosome prepared from rat brain tissue treated with low (0.4 mg/kg) and high (4.0 mg/kg) doses of aldrin. It is clear from the Arrhenius plot (Fig. 1a) that control Na^+,K^+-ATPase did not exhibit TT. The TT changed to 25.2° and 30.0° at low and high doses respectively. Apparent activation energy (AE) of control synaptosomal Na^+,K^+-ATPase was more or less the same

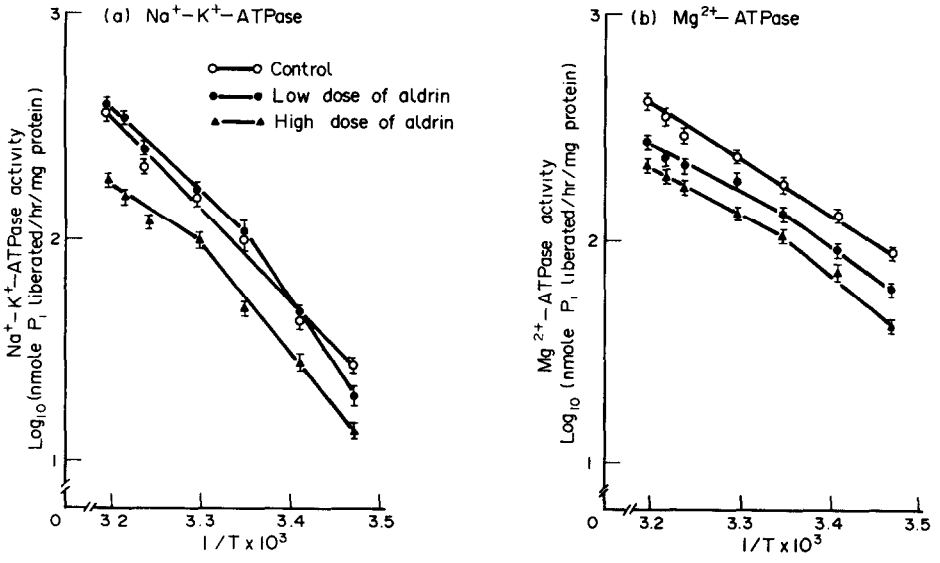

Fig. 1. Arrhenius plots of (a) Na^+-K^+-ATPase and (b) Mg^{2+}-ATPase of brain synaptosomes prepared from rat treated with aldrin (0.4–4.0 mg/kg). Each point represents the mean ± SE of three separate determinations.

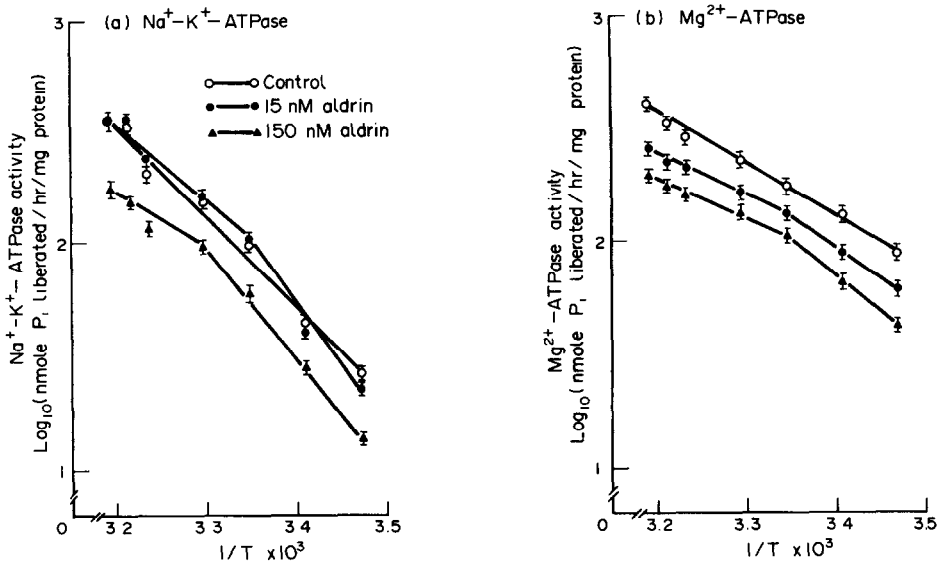

Fig. 2. Arrhenius plots of (a) Na^+-K^+-ATPase and (b) Mg^{2+}-ATPase of rat brain synaptosome in the absence and presence of aldrin (15–150 nM). Each point represents the mean ± SE of three separate determinations.

throughout the temperature whereas AE of synaptosomal Na^+,K^+-ATPase below TT were increased (47.04%, 22.08%) for both the doses of aldrin. AE above TT were decreased (42.32%) at high (4.0 mg/kg) dose of aldrin whereas low (0.4 mg/kg) doses of aldrin did not produce any significant change on AE above TT. It is evident from Fig. 1b that control synaptosomal Mg^{2+}-ATPase did not exhibit TT. TT changed to 25.2° and 25.3° at low and high doses of aldrin respectively. There was no appreciable change of AE noticed in control synaptosomal Mg^{2+}-ATPase. AE below TT was increased (23.34%) at high doses of aldrin. No change in AE was noticed at lower doses of aldrin. AE above TT was decreased (19.91% and 22.09%) at low and high doses of aldrin respectively.

Table 8 represents the result of the Arrhenius plots of Na^+,K^+-ATPase and Mg^{2+}ATPase of synaptosomes in the presence of varying concentrations of aldrin (0–150 nM). It is noted from the Arrhenius

Table 7. *In vivo* effect of aldrin on transition temperature (TT) and apparent activation energy (AE) of synaptosomal Na^+,K^+-ATPase and Mg^{2+}-ATPase

		Na^+-K^+-ATPase				Mg^{2+}-ATPase		
			AE (kcal/mol)				AE (kcal/mol)	
Treatment	Dose (mg/kg, p.o.)	TT°	15°–25°	25°–30°	30°–40°	TT°	15°–25°	25°–40°
Control	—	Nil	19.11 ± 0.38	18.50 ± 0.08	19.21 ± 0.40	Nil	12.38 ± 0.41	11.95 ± 0.53
Aldrin	0.4	25.19 ± 0.39	28.10 ± 0.24*	18.67 ± 1.11	19.34 ± 1.91	25.18 ± 0.40	13.25 ± 0.28	9.57 ± 0.18§
	4.0	30.02 ± 0.53	23.33 ± 0.39†	23.42 ± 0.57*	11.80 ± 0.14*	25.33 ± 0.54	15.27 ± 0.14†	9.31 ± 0.09‡

All values are means ± SE of three separate determinations.
AE was calculated from the slope of the lines of Fig. 1(a) and (b).
Significantly different from control * $P < 0.001$; † $P < 0.005$; ‡ $P < 0.01$; § $P < 0.025$.

Table 8. *In vitro* effect of aldrin on transition temperature (TT) and apparent activation energy (AE) of synaptosomal Na^+-K^+-ATPase and Mg^{2+}-ATPase

		Na^+-K^+-ATPase				Mg^{2+}-ATPase		
			AE (kcal/mol)				AE (kcal/mol)	
Treatment	Concentration (nM)	TT°	15°–25°	25°–30°	30°–40°	TT°	15°–25°	25°–40°
Control	—	Nil	19.18 ± 0.44	18.72 ± 0.28	18.78 ± 0.32	Nil	11.45 ± 0.80	11.65 ± 0.36
Aldrin	15.0	25.39 ± 0.39	28.32 ± 0.38*	19.64 ± 1.22	17.12 ± 0.65	25.60 ± 0.41	13.62 ± 0.33	8.98 ± 0.29†
	150.0	29.85 ± 0.14	23.47 ± 0.44†	23.85 ± 0.43*	12.68 ± 0.56*	25.18 ± 0.39	15.34 ± 0.19‡	8.51 ± 0.34†

All values are means ± SE of three determinations.
AE was calculated from the slope of the lines of Fig. 2(a) and (b).
Significantly different from control * $P < 0.001$; † $P < 0.005$; ‡ $P < 0.01$.

plots (Fig. 2a) that control Na^+,K^+-ATPase did not exhibit a TT whereas this value of TT changed to 25.4° and 30.0° in the presence of 15 nM and 150 nM aldrin respectively. The AE of synaptosomal Na^+,K^+-ATPase below TT were increased (47.65% and 22.05% respectively) in the presence of two concentrations of aldrin (15 nM and 150 nM). AE above TT was decreased (32.48%) in the presence of 150 nm of aldrin whereas aldrin at 15 nM concentration did not produce any significant effect on AE above TT. It is evident from Fig. 2(b) that control synaptosomal Mg^{2+}-ATPase also did not exhibit any TT and the AE were the same throughout the varied temperature. TT changed to 25.6° and 25.2° in the presence of 15 nM and 150 nM aldrin respectively. AE below TT was increased (33.97%) in the presence of 150 nM aldrin but no change of AE was found in the presence of 15 nM of aldrin. AE above TT was decreased in the presence of 15 nM (22.91%) and 150 nM (26.95%) of aldrin.

DISCUSSION

The present study indicates that aldrin is a potent inhibitor of Na^+,K^+-ATPase and Mg^{2+}-ATPase of rat brain synaptosomes. The inhibition of these two ATPases activities under both *in vivo* and *in vitro* conditions by a number of chlorinated hydrocarbons such as DDT, chlordecone etc. have been reported in fish brain [10], rat brain [9, 11, 12, 32] and mouse brain [11]. Results of dialysis experiments (Table 4) and dilution experiments (not shown) suggest that aldrin-induced inhibition of synaptosomal membrane-bound Na^+,K^+-ATPase activity is reversible in nature, whereas that of Mg^{2+}-ATPase is irreversible. Results (Tables 5 and 6) of Lineweaver–Burk plot analyses suggest that aldrin inhibits synaptosomal Na^+,K^+-ATPase activities under both *in vivo* and *in vitro* conditions non-competitively. This indicates that aldrin may bind to the Na^+,K^+-ATPase site which may not be the substrate binding site of this enzyme and modulates the enzymic activity by masking the catalytic site of the membrane-bound enzyme without altering its affinity towards substrates. This non-competitive inhibition induced by other chlorinated hydrocarbons has also been reported by other investigators [9, 33]. The significant inhibition of both V_{max} and K_m with aldrin under *in vivo* and *in vitro* conditions (Tables 5 and 6) suggest that aldrin inhibits Mg^{2+}-ATPase activities uncompetitively.

It is known that phospholipid components of the membrane are extensively involved in the function of the synaptosomal membrane-bound Na^+,K^+-ATPase [34–39], which is known to be an intrinsic enzyme [40, 41]. It is also known that the lipid bilayer [34–39] may be the site of action of aldrin and other chlorinated hydrocarbons on biomembranes. Hence it is not unreasonable to assume that variation of lipid composition of different membranes as well as their structural organization [37–39] may be responsible for the membrane-specific effect of aldrin on Na^+-K^+-ATPase activities. In membrane-bound enzymes it has been observed that there is an apparent sudden change in the enzyme activation energy which may be due to the transition from crystalline to liquid crystalline phase, known as transition temperature (TT) [42]. The temperature variation studies showed that the percentage inhibition of Na^+,K^+-ATPase and Mg^{2+}-ATPase with aldrin was increased with the increase in temperature (Figs 1 and 2). In this context it may be mentioned that K^+-para-nitrophenyl-phosphatase also behaves similarly. Further, it is noted that there is no change in the slope of the Arrhenius plots (TT) of control synaptosomal Na^+,K^+-ATPase and Mg^{2+}-ATPase (Figs 1 and 2). The changes in the slope of the Arrhenius plots of both ATPases in the presence of aldrin could be due to differential inhibition of the enzymes at different temperatures with aldrin. Charnock *et al.* [43] have shown that changes in the slope of Arrhenius plots of Na^+-K^+-ATPase could be due to the varying temperature dependence of two different steps of overall reactions. (Na^+-stimulation of phosphorylation of the phosphorylated enzyme complex and the K^+-induced dephosphorylation of the phosphorylated enzyme complex [44]). The changes in AE for Na^+,K^+-ATPase and Mg^{2+}-ATPase activities over the temperature range of 15–40° (Tables 7 and 8) apparently result from temperature-dependent alterations in K_m [25, 45]. The changes in the kinetic parameters at low and high temperatures observed in the present study in the Na^+,K^+-ATPase and only at high temperature in Mg^{2+}-ATPase could be correlated with the temperature-dependent alterations in the mobility of the membrane lipids which may ultimately regulate the membrane-bound ATPase activities [46]. Thus, finally it may be concluded that aldrin increases the lipid fluidity of the membranes and produces an inhibition of synaptosomal membrane-bound Mg^{2+}- and Na^+,K^+-ATPase activities.

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