ATPase in rat brain under aluminium toxicity

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SUMMARY

The present study deals with evaluation of the impact of aluminium acetate on the ATPase system in rat brain. Male albino rat, *Rattus norvegicus*, weighing 130±2 grams, of 60±2 days age was the experimental animal model and aluminium acetate, the toxicant. Aluminium toxicity (LD50/24h) was evaluated and was found to be 700mg/kg body weight. 1/5th of lethal dose was taken as the sub-lethal dose. For acute dose studies, rats were given a single lethal dose of aluminium acetate orally for one day only and for chronic dose studies, the rats were administered with sub-lethal dose of aluminium acetate once in a day for 25 days continuously. The levels of various constituents of the ATPase system *viz.* Total ATPases, Na^+, K^+ - ATPase and Mg^{+2} ATPase were determined in different regions of rat brain such as Cerebral Cortex (CC), Hippocampus (Hc), Hypothalamus (Ht), Cerebellum (Cb) and Ponsmedulla (Pm) at selected time intervals/days under acute and chronic treatment with aluminium. The results revealed that the levels of all ATPases were inhibited to different extent in all the above areas of brain upon aluminium intoxication thus exhibiting region specific sensitivity of rat brain. While under acute treatment, inhibition in ATPase commenced from 3h, reaching maximum at 12h, under chronic exposure, maximum inhibition was observed on 15th day. Further, in acute treatment, the ATPase system never gained normal levels,where as under chronic exposures, a slight recovery was observed in all ATPases from 20th day onwards and by 25th day almost near normalcy was restored.

Key words : Aluminium acetate, Rat brain; ATPase system, Behavioural changes

Aluminium, the worlds 3rd most common element is widely dispersed in abundance in igneous rocks, shales, clays etc. By virtue of its greatest properties like strength, corrosion resistance, electrical and thermal conductivity, light and heat reflectivity, delibility and formidability, it has an ever increasing number of applications ranging from structural materials to thin packaging foils and electrical transmission appliances. Though dietary aluminium is ubiquitous, in small quantities (30-50mg per day-National Library of Medicine, 2000), it is not a significant source of concern in persons with normal elimination capacity. However, the increase in aluminium exposure of the general population becomes an increasing concern as evidence by the research reports demonstrating possible association between aluminium exposure and chromosomal aberrations (Roy *et al*., 1991), carcinogenicity (Bhamra and Costa, 1992), hypochromic anaemia (Ward, 1991), impairments in motor function (Strong and Garruto, 1991), bone diseases (Querles, 1991) and so many behavioural impairments (Connor *et al.*, 1988). Further, aluminium is known to cause deficits in immune effectors cell function (Golub *et al.*, 1993), lower grip strength and increased startle response (Oteiza, 1993), post-weaning neurobehavioural changes (Donald *et al*., 1989) and increased mortality (Jensen, 1998) in mice.

Besides, the above effects on various organs and their functions, aluminium is also known to exert its toxic effects on the nervous system as well such as degeneration of astrocytes (Suarez-Fernandez, 1999); interfering with the metabolism of the neuronal cytoskeleton (Van der Voet *et al.*, 1999); encephalopathy in dialysis patients (Morris, 1989) and implicated in a series of neurological diseases such as amylotropic lateral sclerosis, dementia associated with Parkinson's disease etc. (Altmann *et al.*, 1999).

In view of the above observations, in the present analysis an attempt has been made to evaluate the toxic effects of aluminium on the ATPase system in the brain of rat subjected to chronic and acute treatment and manifestation of these changes in the behaviour of rat. The reason behind selecting the ATPase system is that the ATPase system plays multiple roles *viz.* as energy transducers (Takao, 1985), as integral membrane proteins facilitating the movement of solutes across the membranes (Boyer, 1976), excitability of neurons (Bonting, 1970), impairing brain energy balance etc. The

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research out come from this present investigation will be of immense value in relating the energy metabolism under aluminium toxicity to neurological diseases in mammalian system.

MATERIALS AND METHODS

Male albino rats, *Rattus norvegicus*, of weighing 130±2 grams, 60±2 days age obtained from Sri Venkateswara Enterprises, Bangalore were selected as experimental animals and Aluminium acetate as the toxicant. The rats were fed with food pellets (Sri Venkateswara Enterprises, Bangalore) and drinking water *ad libitum*. The animals were housed in polypropylene cages under hygienic conditions with photoperiod of 12 hours light and 12 hours dark.

Parameters studied:

Toxicity evaluation:

Probit method of Finney (1964).

ATPase system:

Total ATPases, Na^+, K^+ - ATPase and Mg^{+2} ATPase : (Tirri et al., 1973).

As the above biochemical estimations were done under both acute and chronic exposures. For acute exposures, the animals were sacrificed at 1h, 3h, 6h, 12h and 24h intervals after oral administration of a single lethal dose of aluminium acetate and for chronic exposures, the animals were treated with sub-lethal doses of aluminium acetate every day up to 25th day and sacrificed on 5th day, 10th day, 15th day, 20th day and 25th day. After cervical dislocation, the brain was isolated quickly and placed in ice. Different areas of the brain such as Cerebral Cortex (CC), Hippocampus (Hc), Hypothalamus (Ht), Cerebellum (Cb) and Ponsmedulla (Pm) were isolated by following standard anatomical marks (Glowinski and Iverson, 1966) and were immediately homogenized in suitable media for biochemical analysis. The results obtained were analyzed statistically by following standard methods.

Behavioural studies:

As a corollary to the above, behavioural changes manifested in rat subjected to both acute and chronic doses of aluminium were recorded to coincide with the time intervals selected for ATPase system.

RESULTS AND DISCUSSION

The results of the present investigation clearly indicate that Aluminium acetate has significantly altered the levels of Total ATPases, Na^+, K^+ - ATPase and Mg^{+2} ATPase in all areas of rat brain such as Cerebral Cortex (CC), Hippocampus (Hc), Hypothalamus (Ht), Cerebellum (Cb) and Ponsmedulla (Pm) under both acute and chronic exposures.

Total ATPases:

The observations showed that the level of total ATPases in control rat brain was highest in Hypothalamus (33.45) followed by Cerebral cortex (30.6), Ponsmedulla (28.06), hippocampus (26.77) and Cerebellum (26.04) (Table 1 and Fig. 1).Under exposure to acute doses of aluminium acetate, the extent of inhibition observed in the levels of Total ATPases in various regions of the rat brain at 12h was as follows.

Hippocampus (8.71%), Hypothalamus (8.42%) and Cerebral cortex (6.65%)

Insignificant inhibition was recorded in the remaining brain regions (Table 1). Similarly, under chronic treatment, all the brain areas showed significant inhibition in total ATPases on 15th day, in the order of Hypothalamus (51.32%) > Cerebral cortex (43.21%) > Ponsmedulla (42.67%) > Hippocampus (39.57%) > Cerebellum (37.75%).

Na^+, K^+ - ATPase:

Na^+, K^+ - ATPase activity was also significantly decreased in all brain areas of rat under acute and chronic exposures against the control. The activity levels of Na^+, K^+ - ATPase in different brain areas of control rats was in the following order.

Hypothalamus > Cerebral cortex > Ponsmedulla > Hippocampus > Cerebellum

(27.16%) (25.52%) (23.07%) (22.67%) (22.02%)

Upon administration of acute doses of aluminium acetate, while Hippocampus (8.93%) and Hypothalamus (8.83%) recorded significant decrease in Na^+, K^+ - ATPase at 12h, other brain regions registered insignificant inhibition (Table 2 and Fig. 2). Under chronic doses, Na^+, K^+ - ATPase showed a similar trend of inhibition as in the case of total ATPases on 15th day.

Mg^{+2} ATPase:

In control rats, the level of Mg^{+2} ATPase was highest in Hypothalamus (6.29%) and least in Cerebellum (4.02%). Similar to total ATPases, Mg^{+2} ATPase activity also showed a decreased level in Hippocampus (8.68%) followed by Hypothalamus (6.69%) and Cerebral Cortex (5.34%) at 12h under acute treatment. However, under chronic treatment, a significant inhibition in Mg^{+2} ATPase was noticed on 15th day, in the order of Hypothalamus

Table 1 : Effect of aluminium acetate on Total ATPases activity (μ moles of Pi formed/mg protein/h) in different regions of rat brain.

	ACUTE								CHRONIC							
	C	6h	C	12h	C	24h	C	5d	C	10d	C	15d	C	20d	C	25d
Cc	31.83 ± 1.3	30.36 * ± 1.3 (-4.62%)	31.12 ± .9	29.05 ± .8 (-6.65%)	30.89 ± .8	28.57 ± .9 (-7.51%)	31.14 ± .8	28.78 ± .8 (-7.58%)	31.02 ± .7	24.81 ± .7 (-20.02%)	32.12 ± .10	18.24 ± 09 (-43.21%)	31.14 ± .09	20.33 ± .10 (-34.71%)	31.11 ± .3	21.28 ± .2 (-31.60%)
Hc	27.06 ± .3	25.05 ± .2 (-7.43%)	26.76 ± .8	24.43 ± .9 (-8.71%)	26.52 ± 1.6	24.62 * ± 1.6 (-7.16%)	26.43 ± .7	24.53 ± .8 (-7.19%)	26.08 ± .8	21.04 ± .8 (-19.33%)	26.71 ± .9	16.14 ± .8 (-39.57%)	26.88 ± .13	18.01 ± .13 (-33.00%)	26.99 ± .1	21.65 ± .2 (-19.79%)
Ht	33.57 ± 1.5	31.89 * ± 1.5 (-5.00%)	33.61 ± .9	30.78 ± .8 (-8.42%)	33.64 ± .9	30.75 ± .8 (-8.59%)	33.99 ± .8	30.44 ± .7 (-10.44%)	33.40 ± .7	25.91 ± .8 (-22.43%)	34.08 ± .3	16.59 ± .2 (-51.32%)	33.68 ± .2	20.97 ± .1 (-37.74%)	34.43 ± .10	26.93 ± .09 (-21.78%)
Cb	26.10 ± .6	25.2 * ± .8 (-3.45%)	25.42 ± 1.6	24.26* ± 1.6 (-4.56%)	26.32 ± 1.6	24.50 * ± 1.6 (-5.91%)	26.58 ± .7	23.77 ± .7 (-10.57%)	26.75 ± .8	21.96 ± .7 (-17.91%)	25.80 ± .2	16.06 ± .1 (-37.75%)	26.38 ± .06	17.76 ± .07 (-32.68%)	26.15 ± .3	20.71 ± .2 (-20.80%)
Pm	28.02 ± .6	27.11 * ± .8 (-3.25%)	28.34 ± 1.6	26.49 * ± 1.6 (-6.53%)	28.32 ± 1.6	27.05 * ± 1.6 (-4.48%)	28.41 ± .8	25.91 ± .9 (-8.81%)	28.76 ± .7	20.93 ± .7 (-27.23%)	28.31 ± .13	16.23 ± 13 (-42.67%)	28.33 ± .3	19.27 ± .2 (-31.98%)	28.39 ± .13	21.31 ± .12 (-24.93%)

Values in parentheses indicate per cent changes from control

Values are mean ± SD of six observations each from tissues pooled from 6 animals Values are significant at $p<0.01$

^ Indicate significance at $p<0.05$ * Not significant

Table 2 : Effect of aluminium acetate on Na^+, K^+-ATPase activity (μ moles of Pi formed/mg protein/h) in different regions of rat brain

	ACUTE								CHRONIC							
	C	6h	C	12h	C	24h	C	5d	C	10d	C	15d	C	20d	C	25d
Cc	26.73 ± 1.1	25.43 * ± 1.1 (-4.86%)	26.06 ± 1.6	24.26 * ± 1.6 (-6.91%)	25.86 ± 1.6	23.96 * ± 1.6 (-7.35%)	25.97 ± .2	24.05 ± .1 (-7.39%)	25.70 ± .13	20.37 ± .13 (-20.74%)	26.77 ± .10	14.65 ± .09 (-45.27%)	25.65 ± .3	16.32 ± .2 (-36.37%)	26.11 ± .06	16.98 ± .07 (-34.97%)
Hc	22.88 ± .6	21.15 ± .5 (-7.56%)	22.73 ± .6	20.70 ± .6 (-8.93%)	22.41 ±1.4	20.73 * ± 1.5 (-7.5%)	22.31 ± .2	20.76 ± .1 (-6.95%)	22.04 ± .2	17.80 ± .1 (-19..24%)	22.46 ± .3	13.27 ± .2 (-40.92%)	22.73 ± .10	14.76 ± .09 (-35.06%)	22.55 ± .2	17.84 ± .1 (-20.89%)
Ht	27.23 ± 1.1	25.90 * ± 1.1 (-4.89%)	27.18 ± .6	24.78 ± .6 (-8.83%)	27.12 ± 1.5	24.7 * ± 1.6 (-8.92%)	27.76 ± .10	24.74 ± .09 (-10.88%)	27.06 ± .1	21.16 ± .2 (-21.8%)	27.77 ± .09	12.73 ± .10 (-54.16%)	27.41 ± .3	16.71 ± .2 (-39.07%)	27.14 ± .13	21.75 ± .13 (-19.86%)
Cb	22.06 ± .6	21.4 * ± .5 (-2.99%)	21.39 ± .6	20.5 * ± .8 (-4.11%)	22.30 ± 1.2	20.81 * ± 1.2 (-6.68%)	22.54 ± .09	20.06 ± .10 (-11.00%)	22.7 ± .06	18.71 ± .07 (-17.58%)	21.78 ± .3	13.32 ± .2 (-38.84%)	22.33 ± .3	14.76 ± .2 (-33.9%)	22.12 ± .10	17.23 ± .09 (-22.1%)
Pm	23.05 ± .6	22.36 * ± .5 (-2.99%)	23.38 ± .9	21.81 * ± .9 (-6.72%)	23.38 ± .6	22.49 * ± .8 (-3.81%)	23.47 ± .13	21.30 ± .13 (-9.25%)	23.81 ± .09	16.88 ± .10 (-29.11%)	23.34 ± .2	13.11 ± .1 (-43.83%)	23.37 ± .2	15.64 ± .1 (-33.08%)	23.41 ± .3	17.20 ± .2 (-26.53%)

Values in parentheses indicate per cent changes from control

Values are mean ± SD of six observations each from tissues pooled from 6 animals Values are significant at $p<0.01$

^ Indicate significance at $p<0.05$ * Not significant

(38.83%) followed by Ponsmedulla (37.22%), Cerebral cortex (32.90%), Hippocampus (32.47%) and Cerebellum (31.84%) (Table 3 and Fig. 3).On comparison, it was obvious that in general the entire ATPase system was suppressed under aluminium toxicity under chronic as well as under acute exposures. Further, it is also evident that the inhibition in ATPase system is several folds more in chronic exposure compared to acute treatment.

Behavioural changes:

The behavioural changes exhibited by the rat exposed to acute and chronic doses of aluminium were recorded at selected time intervals/days to coincide with the time schedules for biochemical estimations. These behavioural changes included adipsia (lack of drinking), aphagia (lack of eating), hypokinesia (reduced locomotor activity), fatigue, seizures, difficulty in breathing, lacrymation, salivation, etc.

The observations in the present research demonstrate that aluminium acetate has induced significant and varied levels of inhibition in ATPases activity in all regions of brain under acute and chronic doses of exposure thus giving an insight that aluminium might be affecting various steps in the energy metabolism. ATPases play a central role in physiological functions of cells as energy transducers by coupling the chemical reaction of adenosine triphosphate hydrolysis to the other energetic processes (Takao, 1985). Not only in mice and rats but also even in teleosts, Heavy metals like lead and cadmium are known to alter the ATPase system (Swarnalatha *et al.*, 1991; Subramanyam, 1991) eventually leadng to impairment of Na^+, K^+ and Ca^{+2} pumping mechanism and finally culminating in cellular dysfunction. Exley (1999) has identified a mechanism which involves potentiation of the activities of neurotrasmitters by the action of aluminium thus bringing about subtle and persistent changes.

Historically, high levels of Al have not engendered concern since it appears that little of ingested Al is absorbed and what is absorbed is rapidly excreted. The absorption of aluminium is poorly understood that both soluble and mucosally associated Aluminium metal binding ligands may regulate the initial uptake (Powell and Whitehead *et al.*, 1999). Several recent studies have demonstrated that co-administration of aluminium with citrate, lactate acetate increase aluminium levels in a variety of organs including brain and diets with sub optimal levels of Cu also result in the deposition of aluminium in the tissues (Lui and Stemmer, 1990).

Aluminium toxicity has been recognized in many settings where exposure is heavy and prolonged and renal function is limited. In patients with osteomalacia, there

Table 3: Effect of aluminium acetate on Mg^{+2} ATPase activity (µ moles of Pi formed/mg protein/h) in different regions of rat brain. Values in parentheses indicate per cent changes from control

	ACUTE						CHRONIC									
	C	6h	C	12h	C	24h	C	5d	C	10d	C	15d	C	20d	C	25d
Cc	5.10 ± .8	4.93 * ± .7 (-3.33%)	5.05 ± .2	4.79 ^ ± .2 (-5.34%)	5.03 ± .3	4.61 ^ ± .3 (-8.35%)	5.17 ± .3	4.73 ^ ± .3 (-8.51%)	5.32 ± .6	4.44 ^ ± .6 (-16.54%)	5.35 ± .8	3.59 ± .7 (-32. 90%)	5.49 ± 1.1	4.01 ^ ± 1.1 (-26. 95%)	5.00 ± .5	4.30 ^ ± .4 (-14.00%)
Hc	4.18 ± .2 (-6.70%)	3.9^ ± .2 (-6.68%)	4.03 ± .2	3.68 ^ ± .2 (-8.68%)	4.11 ±.15	3.89 ^ ±.13 (-5.35%)	4.12 ± .3	3.77 * ± .3 (-8. 50%)	4.04 ± .6	3.24^ ± .6 (-19. 80%)	4.25 ± .8	2.87 ^ ± .7 (-32. 47%)	4.15 ± .6	3.25^ ± .6 (-21. 69%)	4.44 ± .4	3.81^ ± .5 (-14. 19%)
Ht	6.34 ± .3 (-5.52%)	5.99 * ± .3 (-6.69%)	6.43 ± .3	6.00 ± .3 (-6.69%)	6.52 ± .3	6.05 ^ ± .3 (-7.21%)	6.23 ± .3	5.7 ^ ± .3 (-8.51%)	6.34 ± .8	4.75 ± .7 (-25.08%)	6.31 ± .6	3.86 ^ ± .6 (-38.83%)	6.27 ± .15	4.27 ±.13 (-31.90%)	6.29 ± .6	5.18^ ± .8 (-17.65%)
Cb	4.04 ± .2 (-5.94%)	3.8 * ± .2 (-5.94%)	4.03 ± .3	3.75 * ± .3 (-6.95%)	4.02 ± .2	3.69 ^ ± .2 (-8.21%)	4.04 ± .2	3.71 ^ ± .2 (-8.17%)	4.05 ± .6	3.25 ^ ± .6 (-19.75%)	4.02 ± .8	2.74 ^ ± .7 (-31.84%)	4.05 ± .6	3.06 ^ ± .6 (-24.44%)	4.03 ± .3	3.48 ^ ± .4 (-13.65%)
Pm	4.97 ± .2 (-4.43%)	4.75 * ± .2 (-4.43%)	4.96 ± .3	4.68 * ± .3 (-5.65%)	4.94 ± .3	4.56 ^ ± .2 (-7.69%)	4.94 ± .2	4.61 ^ ± .2 (-6.68%)	4.95 ± .6	4.05 ^ ± .6 (-18.18%)	4.97 ±1.1	3.12 ^ ± 1.1 (-37. 22%)	4.96 ± .8	3.63 ^ ± .7 (-26.81%)	4.98 ± .6	4.11 ^ ± .6 (-17.47%)

Values are mean ± SD of six observations each from tissues pooled from 6 animals Values are significant at p<0.01
^ Indicate significance at p<0.05 * Not significant

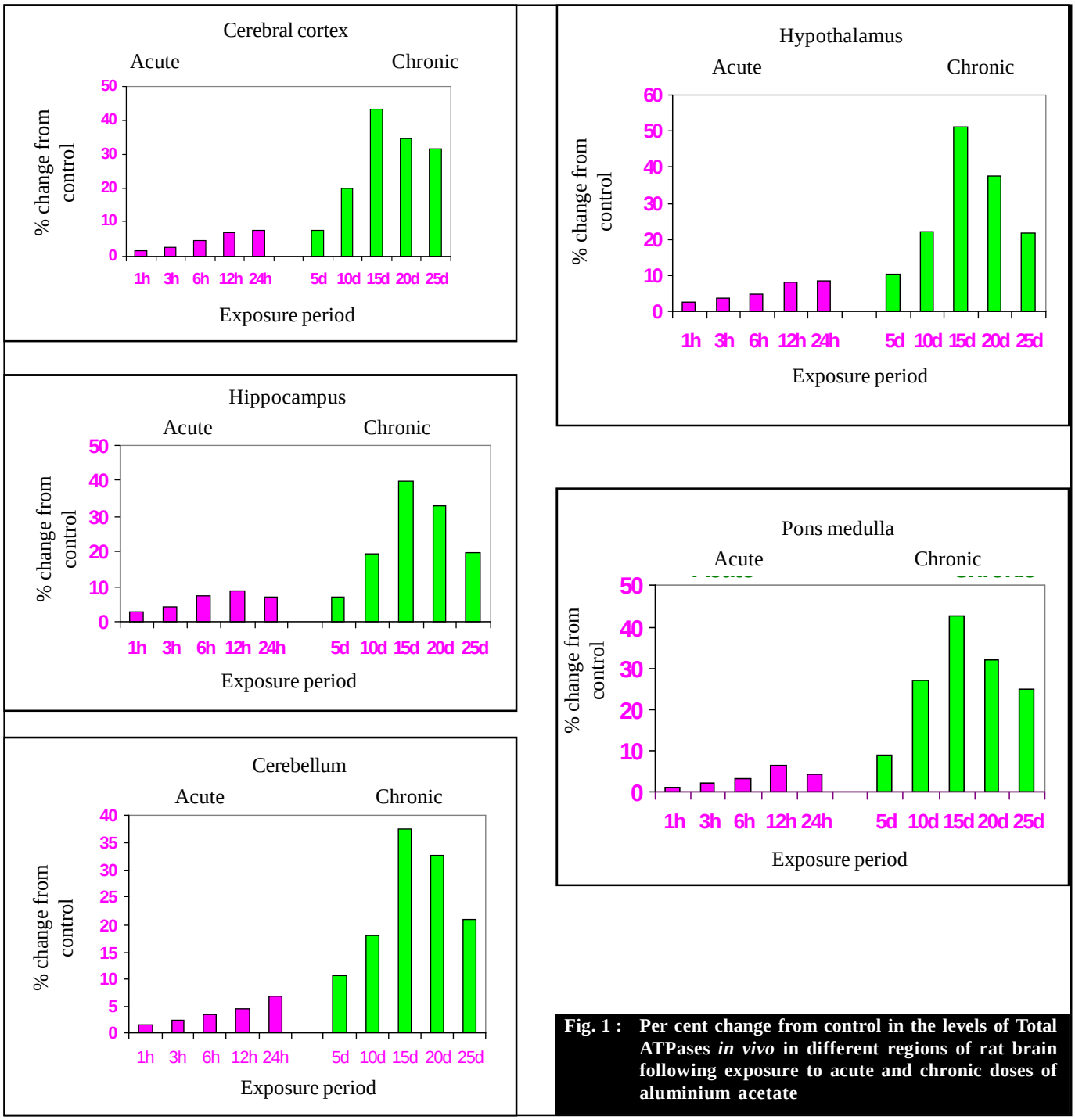

Fig. 1 : Per cent change from control in the levels of Total ATPases *in vivo* in different regions of rat brain following exposure to acute and chronic doses of aluminium acetate

has been a closely associated dialysis encephalopathy caused by aluminium deposition in brain (Suarez *et al.*, 1999). Since the elimination half life of aluminium from the human brain is 7 years, this can result in cumulative damage of the neurons by interfering with neurofilament axonal transport system eventually leading to Alzheimer's like neurofibrillary tangles. The pathogenesis of aluminium toxicity is complex and may be related to other factors such as impaired parathyroid function (Natural Science Library, 2000) and osteomalacia (Kausz *et al.*, 1999; Klein, 1998). Excess aluminium is known to exert direct effect on hematopoiesis, poor immunologic response, physical abnormalities such as stuttering, gait disturbance, myoclonic jerks, seizures, coma, abnormal EEG and sudden death.

Variable levels of inhibition in APases in different brain regions may be due to heterogenous nature of the brain tissue and different roles assigned to them such as striatum-signs of toxicity; cerebellum-cognitive functions;

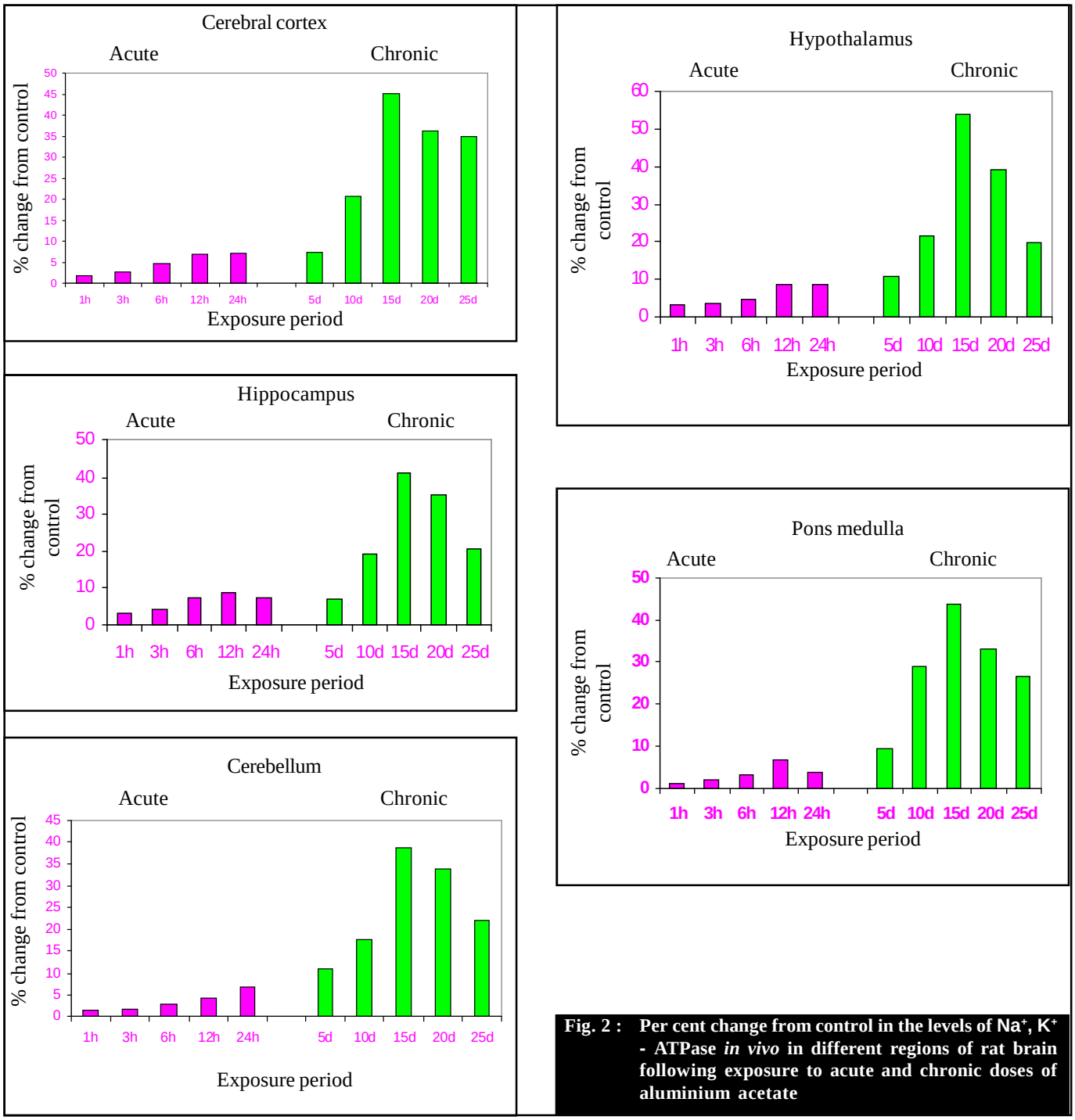

Fig. 2 : Per cent change from control in the levels of Na^+, K^+ - ATPase *in vivo* in different regions of rat brain following exposure to acute and chronic doses of aluminium acetate

hypothalamus-body temperature, thirst emotions; cerebellum-equilibrium; ponsmedulla-respiratory disorders (Cremer *et al.*, 1968). Aluminium was known to accumulate in all regions of the rat brain upon exposure to acute and sub acute doses, maximum accumulation in hippocampus. Further, Aluminium was also seen to compartmentalize in all most all tissues of the body to varying extent, the spleen registering the highest levels (Kandaiah and Kies, 1994; Vasishta and Gill, 1996).Aluminium primarily effects on the cholinergic system and subsequently on non-cholinergic system and finally on ATPase system. Further, recovery tendency noticed in ATPases in all regions of intoxicated rat and its behaviour indicated the operation of the detoxification mechanism and development of behavioural tolerance.

From our present observations, it was obvious that the fluctuations in the ATPases under aluminium toxicity coincided well with the frequency and magnitude of the

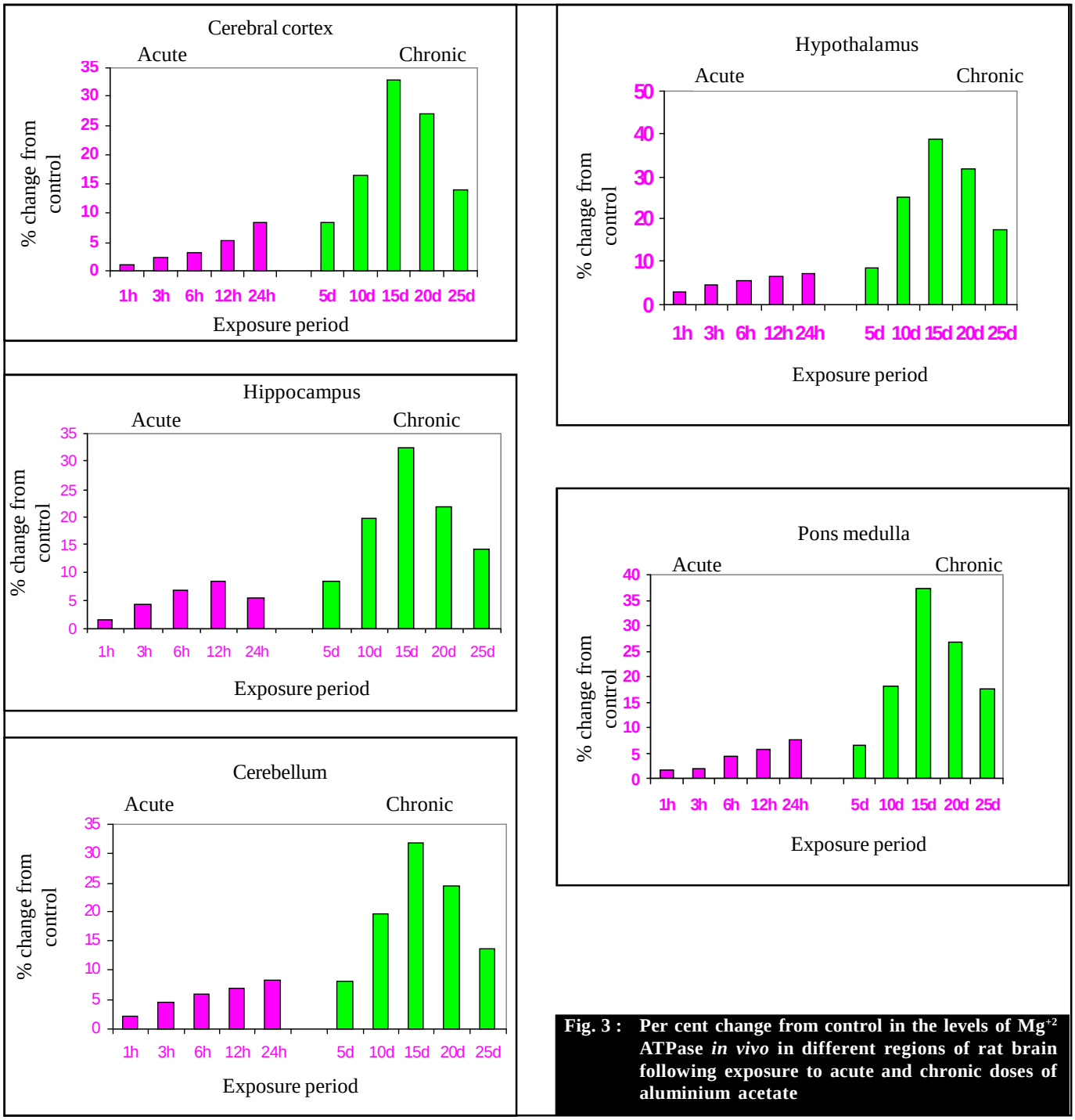

Fig. 3 : Per cent change from control in the levels of Mg^{+2} ATPase *in vivo* in different regions of rat brain following exposure to acute and chronic doses of aluminium acetate

signs and symptoms of several behavioural changes such as adipsia (lack of drinking), aphagia (lack of eating), hypokinesia (reduced movement) etc. Further, these observations support that aluminium might have caused lesions in some of the important regions of brain like substantia nigra, hypothalamus etc. These motor deficits and motivational changes are closely associated with some of the symptoms characteristic to Parkinson's disease (Hewitt *et al.*, 1990). It has been reported that the patients who worked in aluminium smelting industry for 12 years were presented with severe asthma (Vandanplas *et al.*, 1998) and a progressive neurological disorder *viz.*, Potroom palsy with uncoordinated movements, tremors, cognitive deficits etc. (Heyer, 1985). These earlier reports on the relationship between aluminium toxicity and neurodegenerative disorders (Joshi, 1990) in human beings altered its status from being a nontoxic, non absorbable and harmless element to highly toxic heavy

mental.

Regardless of the host and the route of administration, aluminium is proved as a potent neurotoxicant. In the young, adult or developmentally matured host, the neuronal response to aluminium exposure can be dichotomized on morphological grounds, one involving intraneuronal neurofilamentous aggregation and the other producing significant neurochemical and neurophysiological perturbations such as speech disturbances and abnormal EEG, progressive encephalopathy with muscular atrophy, reduced mental development index etc. (Kanwar *et al*., 1996; Strong *et al*., 1996 and Morley *et al*., 1997). Aluminium toxicity is a wide spread problems in all forms of life including humans, animals, fishes, plants and trees causes wide spread degradation of environment and death. Even though aluminium is not considered to be a heavy metal like lead, it can be toxic in excessive amounts and even in small amount if deposited in brain.

The observations in the present investigation provide conclusive evidences that the aspect of aluminium toxicity to human beings needs special attention from the environmentalist point of view since it might increase the risk of occupational hazards with particular reference to neurological diseases.

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