RESEARCH PAPER:

Aluminium toxicity to catecholamines in rat brain

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SUMMARY

The present study demonstrates toxic effects of aluminium on catecholamines of albino rat brain. LD50/ 24h for aluminium as per probit method was 700mg/kg body weight. 1/5th of lethal dose was taken as 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, rats were administered with sub-lethal doses once in a day for 25 days continuously. Various constituents of catecholamines were determined in selected regions of rat brain at selected time intervals and days. The results revealed that the levels of all catecholamines were inhibited differentially in different areas of brain showing region specific response of brain to both modes of exposures to aluminium. However, all these constituents exhibited recovery trend which more pronounced under chronic exposure when compared to acute exposure. Further, these changes in catecholamines were finally manifested in behaviour of rat.

Key words : Aluminium acetate, Rat brain, Catecholamines, Behavioural changes

Aluminium, the worlds 3rd most common element, dispersed in abundance in igneous rocks, shales, clays etc. by virtue of its greatest properties like strength, electrical and thermal conductivity, light and heat reflectivity, delibility and formidability, 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, there is the prolonged exposure and increased mortality (Jensen *et al.*, 1998) in mice.

Further, 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 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, 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 catecholamines in the brain of rat subjected to chronic and acute treatment and manifestation of these changes in the behaviour of rat.

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MATERIALS AND METHODS

Male albino rats, *Rattus norvegicus*, weighing 130±2 g., 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).

– Aminergic system:

Dopamine, Norepinephrine and Epinephrine (Kari *et al.*, 1978).

All 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 (Fig.1) 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 catecholamines.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below :

Dopamine, Norepinephrine and Epinephrine (Fig. 1 to 3, Table. 1, 2 and 3):

The results presented in the above figures clearly indicate that aluminium acetate has significantly altered the levels of Dopamine, Norepinephrine, Epinephrine Monoamine Oxidase in all areas of rat brain such as Cerebral Cortex, Hippocampus, Hypothalamus, Cerebellum and Ponsmedulla under both acute and chronic exposures. All catecholamines showed significant inhibition against the control with acute and chronic exposures of aluminium.

Maximum inhibition in Dopamine was noticed in Hippocampus (28.62%) and least in Hypothalamus (16.99%) at 12 hours during acute dose. Under chronic treatment also, all the brain areas showed significant inhibition in Dopamine after 15 days, and showed highest inhibition in Cerebral Cortex (46.46%) and lowest in Cerebellum (27.45%). After 15 days, Dopamine levels showed recovery through 20 and 25 days (Fig. 1).

Maximum inhibition in Norepinephrine was noticed in Hypothalamus (51.64%) and least in Cerebral cortex (19.46%) at 12 hours during acute dose. Under chronic treatment also, all the brain areas showed significant inhibition in Norepinephrine after 15 days, maximum in Hypothalamus (48.93%) and least in Cerebellum (8.78%) (Fig. 2).

Maximum inhibition in Epinephrine was noticed in Hypothalamus (35.75%) at 24h followed by Cerebellum (33.77%) at 12h, Cerebral cortex (26.88%) at 3h, Ponsmedulla (23.55%) at 6h, and Hippocampus (12.36%) at 6h. Under chronic treatment also, all the brain areas showed significant inhibition in Epinephrine after 15 days, maximum in Hypothalamus (17.56%) and least in

Tablel : Changes in Dopamine content (µg/g wet wt) in different regions of rat brain, exposed to acute and chronic doses of aluminum acetate. Values in parentheses indicate percent changes from control

	Acute										Chronic									
	C	1h	C	3h	C	6h	C	12h	C	24h	C	5d	C	10d	C	15d	C	20d	C	25d
Cc	.386 ± .05	.333* ± .04 (-13.73%)	.384 ± .08	.304* ± .06 (-20.83%)	.383 ± .06	.295 ± .06 (-22.98%)	.387 ± .08	.279 ± .08 (-27.91%)	.389 ± .06	.299 ± .06 (-23.14 %)	.385 ± .06	.299 ± .06 (-22.34 %)	.383 ± .10	.259 ± .09 (-32.38%)	.381 ± .10	.204 ± .12 (-46.46%)	.381 ± .06	.265 ± .08 (-30.45%)	.385 ± .06	.305 ± .06 (-20.98%)
Hc	.548 ± .04	.497* ± .05 (-9.31%)	.546 ± .04	.485^ ± .04 (-10.44%)	.547 ± .06	.443* ± .06 (-19.01%)	.545 ± .12	.389 ± .10 (-28.62%)	.543 ± .04	.473 ± .05 (-12.89%)	.546 ± .06	.429 ± .08 (-21.43%)	.547 ± .12	.379 ± .10 (-30.71%)	.543 ± .15	.328 ± .17 (-39.59%)	.542 ± .10	.376 ± .12 (-30.63%)	.544 ± .06	.423 ± .08 (-22.24%)
Ht	.926 ± .08	.829* ± .08 (-10.48%)	.921 ± .06	.801^ ± .08 (-13.03%)	.928 ± .10	.794* ± .09 (-14.44%)	.924 ± .10	.767 ± .12 (-16.99%)	.923 ± .06	.838 ± .06 (-9.21%)	.928 ± .08	.819 ± .06 (-11.75%)	.925 ± .10	.737 ± .12 (-20.32%)	.923 ± .2	.633 ± .2 (-31.42%)	.928 ± .15	.673 ± .17 (-27.48%)	.925 ± .17	.699 ± .15 (-24.51%)
Cb	.305 ± .02	.283* ± .02 (-7.21%)	.308 ± .03	.264^ ± .03 (-14.29%)	.306 ± .03	.259* ± .03 (-15.36%)	.307 ± .04	.253 ± .04 (-17.59%)	.309 ± .03	.263 ± .03 (-14.89%)	.308 ± .02	.264 ± .04 (-14.29%)	.308 ± .04	.244 ± .05 (-20.78%)	.306 ± .05	.222 ± .06 (-27.45%)	.302 ± .03	.246 ± .03 (-18.54%)	.304 ± .04	.237 ± .05 (-22.04%)
P m	.435 ± .03	.398 * ± .03 (-8.51%)	.439 ± .04	.381 * ± .05 (-13.21%)	.436 ± .04	.362 ± .05 (-16.97%)	.436 ± .04	.361 ± .05 (-17.39%)	.433 ± .04	.379 ± .05 (-12.47%)	.437 ± .04	.374 ± .05 (-14.42%)	.439 ± .06	.322 ± .08 (-26.65%)	.438 ± .10	.278 ± .12 (-36.53%)	.433 ± .06	.322 ± .08 (-25.87%)	.432 ± .08	.349 ± .06 (-19.21%)

Values are mean ± SD of six observaticns each from tissues pooled from 6 animals Values are significant at p<0.01 ^ Indicate significance at p<0.05 * No: significant

Table 2 : Changes in Norepinephrine content (µg/g wet wt) in different regions of rat brain, exposed to acute and chronic doses of aluminum acetate. Values in parentheses indicate percent change from control

	Acute												Chronic							
	C	1h	C	3h	C	6h	C	12h	C	24h	C	5d	C	10d	C	15d	C	20d	C	25d
Cc	1.257 ± .4	.957* ± .5 (-23.87%)	1.250 ± .2	.941^ ± .2 (-24.72%)	1.256 ± .25	.988* ± .3 (-21.34%)	1.254 ± .15	1.010^ ± .13 (-19.46%)	1.253 ± .12	1.099^ ± .10 (-12.29%)	1.252 ± .08	1.159^ ± .06 (-7.43%)	1.259 ± .2	0.969^ ± .2 (-23.03%)	1.257 ± .2	.869^ ± .3 (-30.87%)	1.253 ± .2	.937* ± .3 (-25.22%)	1.252 ± .17	.997^ ± .15 (-20.37%)
Hc	1.229 ± .25	.942* ± .30 (-23.35%)	1.223 ± .2	.895^ ± .2 (-26.82%)	1.224 ± 2	.825^ ± .3 (-32.60%)	1.226 ± 3	.739^ ± .4 (-39.72%)	1.225 ± .10	1.097* ± .12 (-10.45%)	1.225 ± .10	.979^ ± .13 (-20.28%)	1.226 ± .15	.969 ± .13 (-20.96%)	1.224 ± .15	.949^ ± .17 (-22.47%)	1.227 ± .15	.997^ ± .17 (-18.74%)	1.225 ± .15	.999^ ± .13 (-18.45%)
Ht	1.496 ± .8	.823* ± .9 (-44.99%)	1.493 ± .4	.800^ ± .5 (-46.42%)	1.497 ± 4	.759^ ± .5 (-49.30%)	1.495 ± 4	.723^ ± .5 (-51.64%)	1.496 ± .13	.947 ± .3 (-36.70%)	1.496 ± .3	1.247^ ± .15 (-16.48%)	1.498 ± .4	.838^ ± .5 (-44.06%)	1.496 ± .5	.764^ ± .4 (-48.93%)	1.493 ± .3	.899* ± .3 (-39.79%)	1.495 ± .4	1.035^ ± .3 (-30.77%)
Cb	.796 ± .25	.649* ± .25 (-18.47%)	.799 ± .12	.640^ ± .10 (-19.9%)	.798 ± .12	.620^ ± .12 (-22.31%)	.797 ± .12	.604^ ± .10 (-24.22%)	.795 ± .10	.664^ ± .09 (-16.48%)	.793 ± .10	.775^ ± .01 (-2.27%)	.795 ± .02	.759^ ± .02 (-4.53%)	.797 ± .04	.727^ ± .05 (-8.78%)	.796 ± .04	.735^ ± .05 (-7.65%)	.798 ± .03	.749^ ± .03 (-6.14%)
Pm	2.729 ± .5	2.149 * ± .6 (-21.25%)	2.726 ± .5	2.056^ ± .5 (-24.58%)	2.723 ± 5	1.888 ± .4 (-30.66%)	2.725 ± 6	1.564 ^ ± .8 (-42.61%)	2.724 ± .4	2.003^ ± .5 (-26.47%)	2.724 ± .4	2.337^ ± .3 (-14.11%)	2.725 ± .4	1.949^ ± .5 (-28.48%)	2.727 ± .8	1.787 ^ ± .6 (-34.47%)	2.726 ± .4	1.889 * ± .5 (-30.7%)	2.728 ± .2	2.507 * ± .3 (-8. 10%)

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 3 : Changes in Epinephrine content (µg/g wet wt) in different regions of rat brain, exposed to acute and chronic doses of aluminum acetate. Values in parentheses indicate per cent changes from control

	Acute												Chronic							
	C	1h	C	3h	C	6h	C	12h	C	24h	C	5d	C	10d	C	15d	C	20d	C	25d
Cc	.559 ± .09	.429* ± .15 (-23.26%)	.558 ± 08	.408^ ± .12 (-26.88%)	.559 ± .08	.469* ± .09 (-16.10%)	.557 ±.08	.492* ± .10 (-11.67%)	.555 ± .07	.508* ± .09 (-8.47%)	556 ± .04	.510* ± .05 (-18.27%)	.551 ± .03	.500 ± .02 (-9.26%)	.553 ± .03	.497^ ± .05 (-10.13%)	.554 ± .07	.502* ± .09 (-9.39%)	.552 ± .04	.522* ± .05 (-5.43%)
Hc	.729 ± .08	.655* ± .09 (-10.01%)	.721 ± 06	.642^ ± .03 (-10.96%)	.728 ± .06	.638^ ± .07 (-12.36%)	.650 ± .06	.726* ± .07 (-11.69%)	.663 ± .04	.723 ± .02 (-9.05%)	720 ± .02	.695* ± .03 (-3.47%)	.726 ± .04	.666 ± .02 (-8.26%)	.721 ± .04	.648^ ± .05 (-10.12%)	.725 ± .04	.689* ± .05 (-4.97%)	.723 ± .02	.715* ± .01 (-1.11%)
Ht	.895 ± .07	.825* ± .08 (-7.82%)	.893 ± 07	.790^ ± .05 (-11.53%)	.897 ± .15	.750* ± .13 (-16.39%)	.893 ± .09	.729^ ± .12 (-18.37%)	.895 ± .15	.575 ± .18 (-35.75%)	893 ± .04	.844* ± .05 (-5.49%)	.897 ± .09	.797* ± .10 (-11.15%)	.894 ± .12	.737^ ± .10 (-17.56%)	.892 ± .09	.757^ ± .10 (-15.13%)	.891 ± .10	.787* ± .09 (-11.67%)
Cb	.388 ± .04	.354* ± .05 (-8.75%)	.385 ± 07	.333* ± .09 (-13.51%)	.381 ± .08	.308* ± .08 (-19.16%)	.382 ± .07	.253^ ± .06 (-33.77%)	.383 ± .06	.326* ± 07 (-14.88%)	389 ± .03	.358* ± .04 (-7.97%)	.387 ± .04	.349* ± .05 (-9.82%)	.388 ± .02	.339^ ± .04 (-12.63%)	.386 ± .04	.347* ± .07 (-10.10%)	.385 ± .02	.380* ± .01 (-1.30%)
Pm	.775 ±. 09	.673 * ± .13 (-13.16%)	.779 ± 12	.632^ ± .06 (-18.87%)	.777 ± .11	.594^ ± .13 (-23.55%)	.775 ± .14	.666* ± .13 (-14.06%)	.776 ± .08	.684* ± .10 (-11.86%)	777 ± .08	.698* ± .08 (-10.17%)	.775 ± .07	.666^ ± .05 (-14.06%)	.774 ± .10	.639^ ± .09 (-17.44%)	.772 ± .10	.655* ± .09 (-15.16%)	.776 ± .08	.697* ± .06 (-10.18%)

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

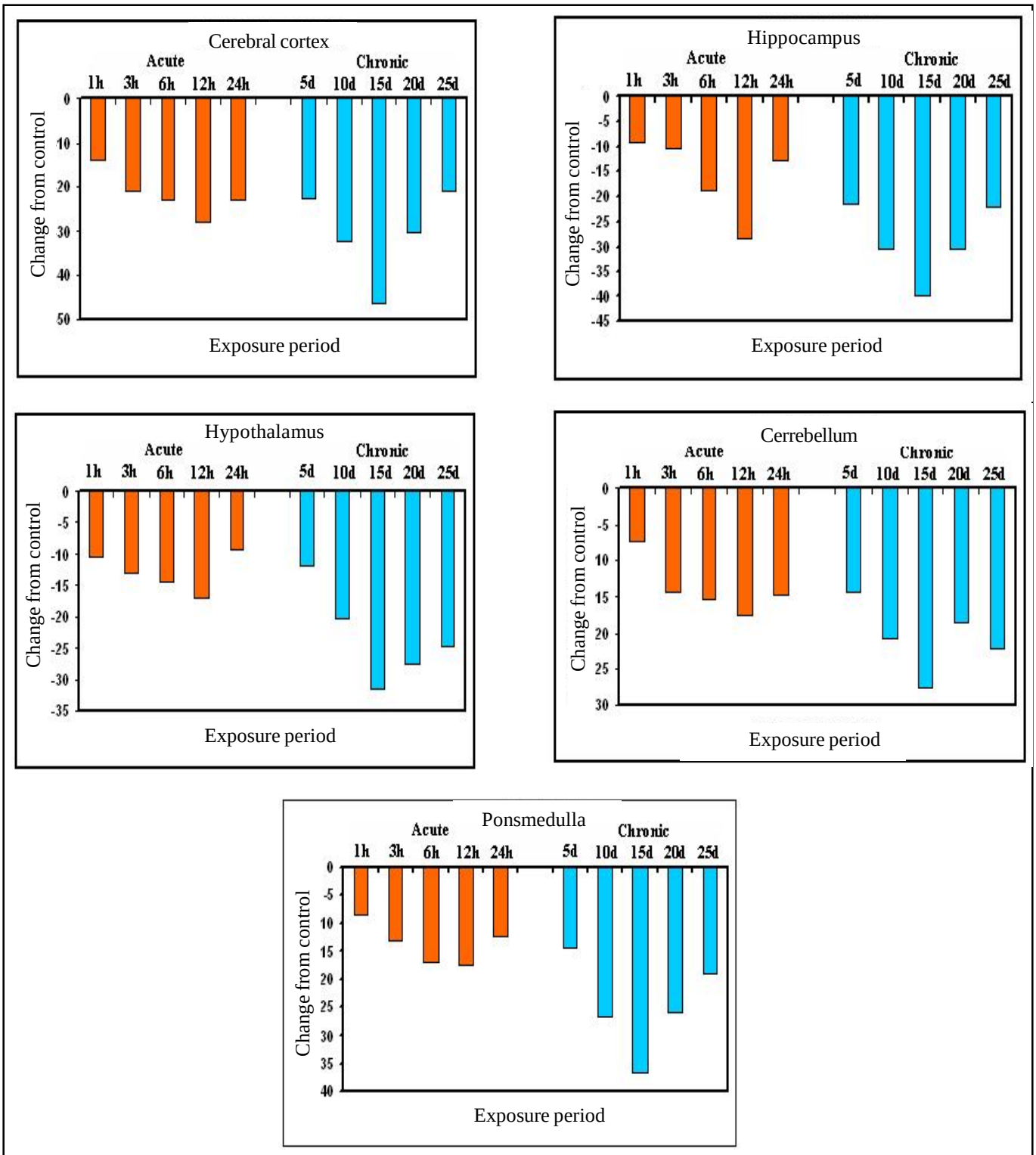

Fig. 1 : Per cent change in *in vivo* content of Dopamine in various regions of rat brain following exposure to acute and chronic doses of aluminium acetate

Hippocampus (10.12%) (Fig. 3).

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

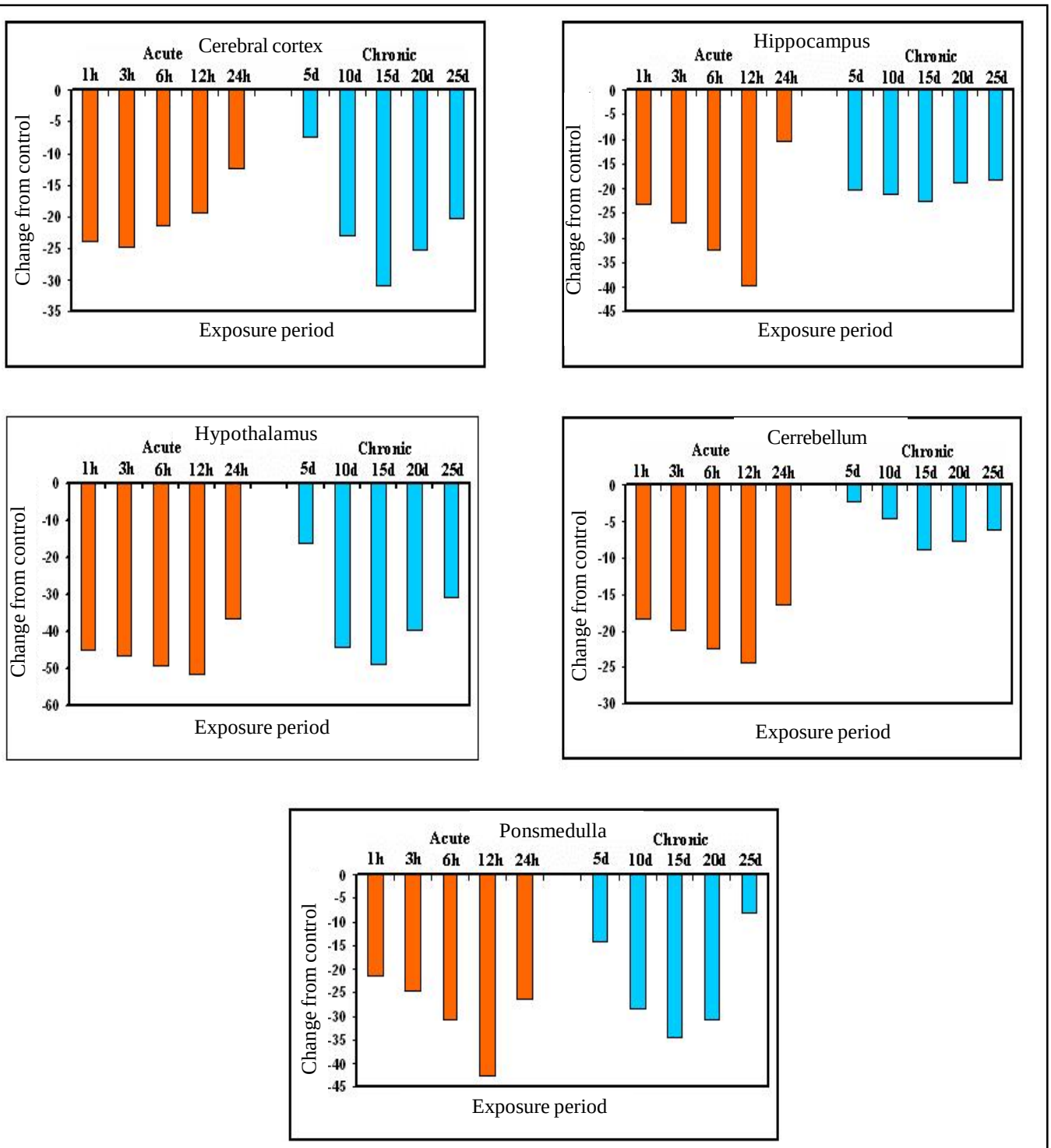

Fig. 2 : Per cent change in *in vivo* content of Norepinephrine in various regions of rat brain following exposure to acute and chronic doses of aluminium acetate

of eating), hypokinesia (reduced locomotor activity), fatigue, seizures, difficulty in breathing, lacrymation, salivation, etc.

The observation in the present study emphasize that aluminium acetete has induced significant and varied levels of inhibition in catecholamines in various regions of rat brain under both acute and chronic exposures. These results substantiate that aluminium might be affecting various steps in the metabolic pathway of the synthesis of these neurotransmitters via end-product inhibition which is maximal when neuronal activity and transmitters release are low, there by leading to high

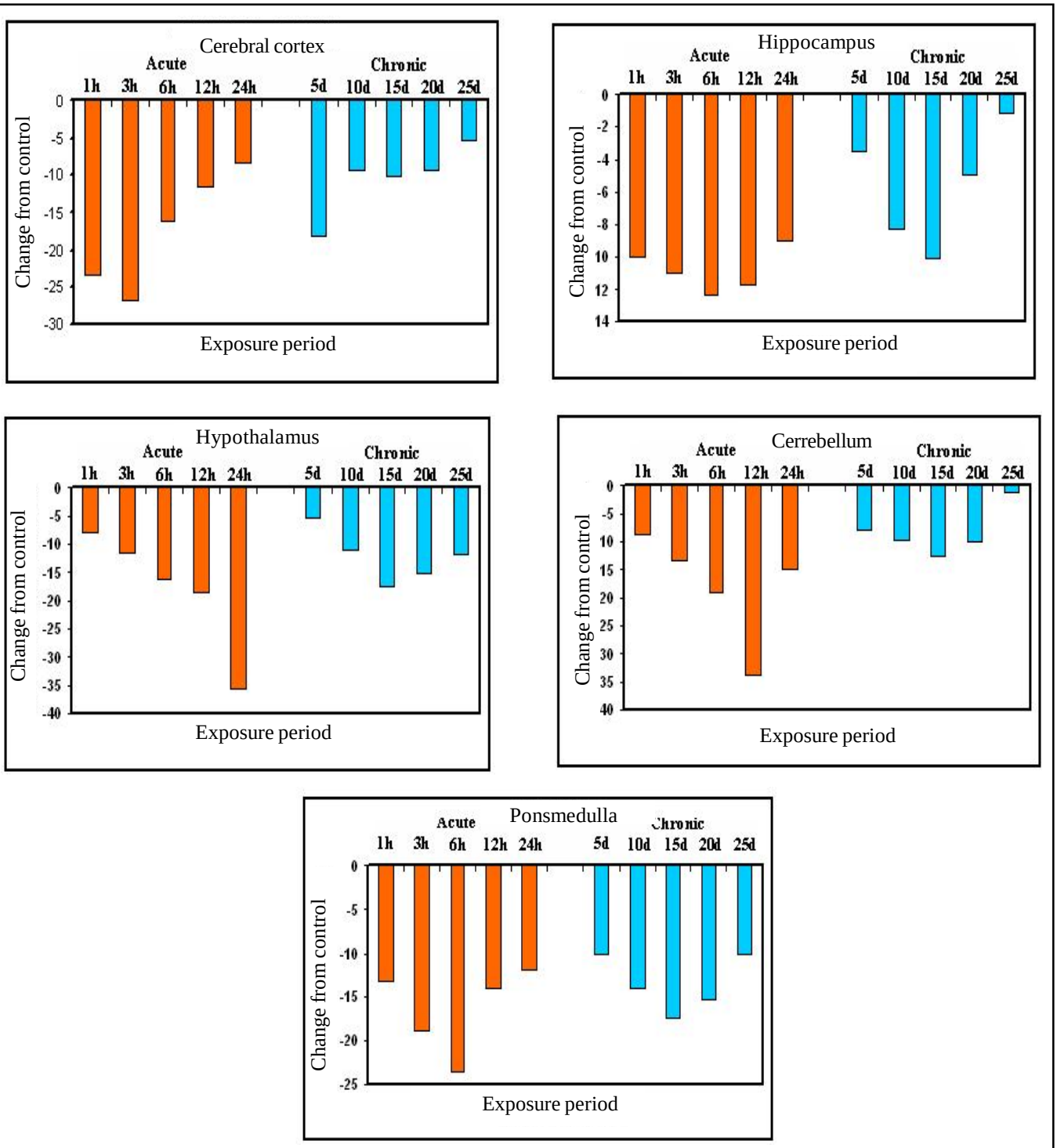

Fig. 3 : Per cent change in *in vivo* content of Epineprine in various regions of rat brain following exposure to acute and chronic doses of aluminium acetate

catecholamine concentration in Tyrosine Hydroxylase (TH) accessible pool (Masserano, 1989).

The synthesis of aminergic neurotransmitters is regulated by a bewildering variety of processes, many of which operate via the rate limiting enzyme Tyrosine Hydroxylase. Some of the factors that regulate the synthesis of the neurotransmitters operate very rapidly thereby allowing cells to respond to short term needs. It should also be noted that studies on the control of these neurotransmitters synthesis have used a number of different model system, including adrenal medullary chromaffin cells, pheochromocytoma cells, sympathetic

noradrenergic neurons, noradrengergic neurons of the locus coeruleus, and nigrostriatal dopaminergic neurons (Feldman *et al.*, 1997). In spite of the wide range of effects possible, those processes which regulate pre-synaptic transmitter release appears to be the most sensitive to heavy metals which bind to calcium ions causing blocking of transmission at the synapse (Howell *et al.*, 1984).

MAO is located on the outer membranes of the mitochondria, to catabolize catecholamine molecules in the nerve terminal cytoplasm. MAO is not only present in noradrenergic and dopaminergic cells, but also plays a central role in the metabolism of aminergic neurotransmitters. Cloning studies have recently identified the specific rat and human vesicular transporter protein for noradrenergic, dopaminergic, and serotonergic cell groups in the brain, indicating that this one protein is probably responsible for accumulating all three amines in synaptic vesicles.

Variable levels of inhibition in these catecholamine neurotransmitters in different brain regions were due to heterogenous nature of the brain tissue and different roles assigned to different neurotransmitters such as Norepinephrine and Serotonin-motor hyper activity (Au and Rabinson, 1988), Dopamine- complex stereotypy (Kulkarni and Dandia, 1972) or due to the disturbances in the cholinergic system (Barkowska *et al.*, 1980). However, the effects of AChE inhibitors on Monoamine oxidase levels in rat brain are confusing (Fosbracy *et al.*, 1990). The areas of rat brain exhibiting changes in cholinergic system are shown to exhibit the greatest changes in non-cholinergic system (Fosbracy *et al.*, 1990) thus indicating their possible interdependence. It is well known fact that the cholinergic and non-cholinergic system are interlinked in the central nervous system (Vizi *et al.*, 1981; Lehmann and Langer, 1983). Thus, it is conceived that the adaptive changes underlying tolerance to anticholinesterase agents involve alterations in other neurotransmitter systems as well in balance with the cholinergic system.

The behavioural changes such as adipsia (lack of drinking), aphagia (lack of eating), hypokinesia (reduced movement) etc. observed in rats under Aluminium toxicity revealed that aluminium might have caused leations in the important regions of brain like substantial nigra, hypothalamus etc. These motor deficits and motivational changes are closely associated with some of the symptoms characteristic to Parkinson's disease (Marshall and Teitelbaum, 1974). The observations in the present study give clear indications that continuous exposure to aluminium compounds for prolonged durations might increase the risk of Alzhiemer disease and Parkinson's disease among the working community in the industries. The noradrenergic neurons, located in the pons and medulla modulate a variety of important behavioural and physiological processes.

The observations in the present study provide conclusive evidences that the aspect of aluminium toxicity to human beings needs special attention from the environmentalist point of view to suggest proper precautionary measures to be implemented in working industries since, prolonged exposure of human beings to aluminium compounds poses higher risk of occupational hazards.

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