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ORIGINAL ARTICLE

Effect of Aluminium and Copper on Dopamine Synthesis in Striatal Synaptosomes of Rat's Brain

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ABSTRACT
Considerable evidence has accumulated indicating involvement of metal ions in the pathophysiology and pathogenesis of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and prion disease. In this study, we investigated the effects of aluminum and copper ions on the amount of dopamine synthesis in rat brain striatal synaptosomes. 40 Male Wistar rats randomly divided into two treated and control groups. Animals were sacrificed by decapitation followed by rapid removal of the brain. The brain was immediately frozen in liquid nitrogen. Striatum samples were removed whole by free-hand dissection. brain striatum homogenized and centrifuged at 1000 rpm. Concentration of extracted dopamine was assayed spectrophotofluorimetrically The results demonstrated that neurological toxicity of aluminum could be due to its inhibitory effect on dopamine synthesis as the chronic treatment with aluminum ions significantly down-regulated the dopamine production level. In addition, the obtained results indicated that acute treatment with copper ions significantly increased the amount of dopamine synthesis. However, acute aluminum treatment and chronic treatment with copper ions did not show any significant effect. These results show the neurotoxic effects of metal ions on neurological system and could provide promising targets for new therapy strategies.
Keywords: *Synaptosome, Dopamine Synthesis, Rat Brain, Striatum, Aluminum ion, Copper ion, Neurological Toxicity*

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INTRODUCTION

Epidemiological and pathological studies suggest a role for aluminum in neurological disorders like Alzheimer's disease and Parkinson's disease [1]. Therefore, aluminum-induced biochemical changes that lead to behavior and pathological disorders have become more interesting for researchers. A number of enzymes and pathways have been introduced as aluminum targets based on its biological effects on central nervous system.

In extra-pyramidal motor activity disorders such as Parkinson's disease, dopamine and norepinephrine concentrations and also activity levels of their synthesizing enzymes decrease in basal ganglia and some other regions of brain like hypothalamus. In addition, concentrations of copper ions are higher than zinc in brain and plasma of these patients [2].

There is extensive evidence for the association of protein aggregation and neurodegeneration in many disorders such as prion disease. It is now evident that metals like copper play a crucial role in protein aggregation and therefore might provide a link between the two processes of protein aggregation and neurodegeneration [3].

Moreover, it has been demonstrated that copper aggregates in basal ganglia in Wilson's disease interfering the metabolism of amines in cortex which it leads to extra-pyramidal neurological symptoms. It is suggested that alteration in catecholamines concentrations is responsible for neurological symptoms in this type of disorders [4]. On the other hand, concentrations of catecholamines are mostly regulated by the activity levels of their synthesizing enzymes. One such enzyme is dopamine β-hydroxylase that catalyzes the final stage of norepinephrine synthesis from dopamine in the both medulla vesicles of the adrenal glands and synaptic vesicles of noradrenergic neurological system. It is a metal enzyme that

copper ion plays a key role for its activity. Studies have indicated that a specific concentration of copper ion inhibits the activity of the enzyme [4].
Furthermore, it has been shown that there are endogenous inhibitors in contribution with copper ion and dopamine β-hydroxylase enzyme in the adrenal glands and brains' medulla vesicles [5]. It has been demonstrated that norepinephrine synthesis is under regulation of these inhibitors in various regions of brain at the dopamine β-hydroxylase level. These endogenous inhibitors contain sulfhydryl compounds and glutathione [6,7].
Dopamine is one of the most important catecholamine neuro-transmitters in the mammalian central nervous system. Several important diseases of the nervous system are associated with dysfunctions of the dopamine system. In the current study, we evaluated the effects of different concentrations of aluminum and copper ions in various time-points on dopamine synthesis in rat brain striatal synaptosomes as the study model.

MATERIALS AND METHODS

Synaptosomes Preparation

40 Male Wistar rats (Razi Institute, Karaj, Iran) aged 12 weeks and 200-350 g weights were used. Animals had free access to food and water and 12hr dark/light time cycles were provided. They randomly divided into 2 groups including treated and control groups. Animals were sacrificed by decapitation followed by rapid removal of the brain. The brain was immediately frozen in liquid nitrogen. Striatum samples were removed whole by free-hand dissection. All animals were injected intra-peritoneal and treated in accordance with the National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals. The extraction of synaptosomes from rats' brain striatum was done according to method published by Messripour and Clark [8]. Briefly, brain striatum homogenized and centrifuged at 1000 rpm. Supernatant containing synaptosomes was removed and stored at -80°C until protein extraction.

Dopamine Extraction

After acid washing of extracted synaptosom, the solution was centrifuged and supernatant was transferred to a new tube containing Tris buffer (N-tris-(hydroxymethyl)-aminomethane, pH 7.4 at 25°C) (Sigma; USA). After 2min incubation on ice, solution was centrifuged and pellet was washed twice. Pellet was suspended in 1ml ethanol and stored at -80°C.

Detection of Dopamine Concentration

Concentration of extracted dopamine was assayed spectrophotofluorimetrically [9]. Three 'standards' and 3 'reagent blanks' were prepared, together with samples internal standards and unoxidized tissue blanks. The whole procedure was carried out in Silica test tubes. After each addition and after irradiation the contents of the tubes being completely mixed on a vortex mixer. After the final acidification the silica tubes, in a transparent methacrylate rack were placed horizontally 15 cm below a u.v. lamp. Fluorescence was measured within 1h on a spectrophotofluorimeter at activation/fluorescence wavelengths of 330/375 nm.

RESULTS

Effects of aluminum-chloride on dopamine synthesis

Chronic treatment of rats with aluminum-chloride significantly decreased the amount of dopamine synthesis in their brain striatal synaptosomes. This effect was dose-dependent and also time-dependent as increase in dosage and/or duration of treatment enhanced the effects of aluminum-chloride.
As shown in table 1, after 2 mg/kg dose of aluminum-chloride injection, dopamine synthesis decreased 28, 68.3, and 79.51% in comparison with control group after 15, 30, and 60 days respectively. After treatment with 7.5 mg/kg dose of aluminum-chloride, the amounts of dopamine reduction were 59, 77.77, and 93% after 15, 30, and 60 days respectively. After 15, 22, and 52 days of treatment with 10 mg/kg aluminum-chloride, the levels of dopamine production decrease were 64.7, 83, and 93.5% respectively.
It was observed that rats with 350 g weight were more sensitive to treatment with 10 mg/kg dose of aluminum-chloride compared to rats with 250 g weight, as 12 rats (350 g weight) died after 1 week treatment with 10 mg/kg aluminum-chloride.
The results did not show any significant difference in acute treatment of rats with 20 mg/kg aluminum-chloride for 24 h between control (8.461 ± 0.19) and treated (8.621 ± 0.129) groups.

Effects of copper-sulfate on dopamine synthesis

As shown in table 2, chronic treatment of rats with 1.75 mg/kg copper-sulfate (for 1 day, 1 week, and 1 month) did not show any significant effect on dopamine production. However, acute treatment of rats with copper-sulfate significantly increased the amount of dopamine synthesis (Table 3).

The results exhibited that copper-sulfate injection with 3. 5. And 10 mg/kg doses 45, 75, and 83% increased the level of dopamine synthesis after 15 min and 37, 58, and 64% increase was observed after 1 h and 18, 28, and 35% up-regulation was detected after 4 h, respectively, in comparison with control group.

Table 1. The effects of aluminum-chloride on dopamine synthesis in rat brain synaptosomes shown as Mean ± SEM.

Dosage	Period	15 days (µg/mg)	30 days (µg/mg)	60 days (µg/mg)
2 mg/kg	Control Group	8.621 ± 0.129	7.907 ± 0.414	8.776 ± 0.545
	Treated Group	6.220 ± 0.219	2.824 ± 0.173	1.798 ± 0.036
	Mean Change (%)	28*	63.8*	79.51*
7.5 mg/kg	Control Group	8.621 ± 0.129	8.907 ± 0.414	8.776 ± 0.545
	Treated Group	3.534 ± 0.214	1.980 ± 0.012	0.682 ± 0.063
	Mean Change (%)	59*	77.77*	93*
10 mg/kg	Control Group	8.621 ± 0.129	8.907 ± 0.414	8.776 ± 0.545
	Treated Group	2.234 ± 0.212	0.905 ± 0.010	0.452 ± 0.060
	Mean Change (%)	64.7*	83*	93.5*

*$p<0.05$

Table 2. The effects of copper-sulfate on dopamine synthesis in rat brain synaptosomes shown as Mean ± SEM.

Dosage	Period	1 day (µg/mg)	1 week (µg/mg)	1 month (µg/mg)
1.75 mg/kg	Control Group	8.621 ± 0.129	8.907 ± 0.414	8.776 ± 0.545
	Treated Group	9.495 ± 0.764	8.440 ± 0.057	10.301 ± 1.721
	Mean Change	NS	NS	NS

NS; not significant

Table 3. The effects of copper-sulfate on dopamine synthesis in rat brain synaptosomesin shown as Mean ± SEM.

Dosage	Period	15 minute (µg/mg)	1 hour (µg/mg)	4 hours (µg/mg)
3 mg/kg	Control Group	8.621 ± 0.129	8.907 ± 0.414	8.776 ± 0.545
	Treated Group	11.001 ± 0.263	12.103 ± 0.590	10.021 ± 0.737
	Mean Change (%)	45*	37*	18*
5 mg/kg	Control Group	8.621 ± 0.129	8.907 ± 0.414	8.776 ± 0.545
	Treated Group	14.736 ± 0.660	13.358 ± 0.55	10.27 ± 0.403
	Mean Change (%)	75*	58*	28*
10 mg/kg	Control Group	8.621 ± 0.129	8.907 ± 0.414	8.776 ± 0.545
	Treated Group	20.261 ± 0.154	16.112 ± 1.366	13.13 ± 0.914
	Mean Change (%)	83*	64*	35*

*$p<0.05$

DISCUSSION

Aluminum has been an interesting topic to investigators for years because of its neurotoxic effects. In this study, we assessed the chronic and acute effects of aluminum treatment on dopamine synthesis in rat brain striatal synaptosomes. The results indicated a significant inhibitory effect of aluminum during a chronic injection. Moreover, this effect was dose- and time-dependent as increase of dosage of aluminum in specific time duration and also increase of treatment time with a specific dosage of aluminum increased the inhibitory effect on dopamine synthesis. However, acute treatment of rats with aluminum did not show any significant effect on the amount of dopamine production. It can be assumed that aluminum was aggregated in brain in a chronic manner probably to show its neurotoxic effects.

Studies have demonstrated that aluminum blocks BH_4 synthesis *in vitro* and addition of transferrin, a strong aluminum-chellator, to the media can reverse this effect [10]. Since BH_4 is the co-enzyme for tyrosine hydroxylase, the key enzyme of dopamine synthesis pathway, it could be possible that inhibitory effect of aluminum on dopamine production is through this mechanism.

In addition, it has been exhibited that aluminum interacts with transferrin [1, 11] and studies have demonstrated that the transferrin-binding aluminum decreases the intake of iron and manganese through down-regulating the surface receptors of oligodendrocytes [12, 13]. Since tyrosine hydroxylase is a metal enzyme containing iron, reduction in iron intake could interfere the activity of enzyme and inhibit the synthesis of dopamine.

Moreover, researchers have shown that neurotoxic effects of aluminum could also be due to its interactions with specific lipids of cell membrane [14]. The mechanisms for metal transition ions promoted lipid peroxidation are H_2O_2 decomposition and direct homolysis of endogenous hydroperoxides. The Fe^{2+}-H_2O_2-mediated lipid peroxidation takes place by a pseudo-second order process, and the Cu^{2+}-mediated process by a pseudo-first order reaction. At low level of H_2O_2, Fe^{2+} induces lipid peroxide decomposition, generating peroxyl and alkoxyl radicals and favoring lipid peroxidation. These results indicate that the onset of the Fe^{2+} stimulatory effect on Fe^{2+}-dependent lipid peroxidation is due to reactive oxygen species production via Fe^{2+} oxidation with endogenous ROOH [15]. It can be stated that aluminum mimics iron effects on lipid peroxidation in cell membrane and could result in destroying the neuron and down-regulating the production of dopamine.

Another possible mechanism for the inhibitory effect of aluminum on dopamine synthesis that we reported in current study could be through the inhibitory effect of aluminum on calcium influx into synaptosomes that Koenig and Jope [16] have shown before. Since calcium as a secondary messenger plays a critical role in release of dopamine, blocking the influx of calcium into synaptosomes could inhibit the release of dopamine.

There is evidence suggesting that there are endogenous inhibitors in association with copper ion and dopamine β-hydroxylase enzyme in medulla vesicles of the adrenal glands and also in brain (Hegde and Friday 1998). It has been demonstrated that norepinephrine synthesis is under regulation of these inhibitors in various regions of brain at the dopamine β-hydroxylase level. These endogenous inhibitors are contained sulfhydryl compounds and glutathione [6, 7]. According to these findings, soluble copper ions in the vesicles could interact with the endogenous inhibitor and reverse the enzyme inhibition which depends on soluble copper ions concentrations in the vesicles as if the concentrations of soluble ions were higher than the sulfhydryl inhibitors', then copper ions could inhibit the dopamine β-hydroxylase activity. Since the amounts of norepinephrine and dopamine β-hydroxylase activities are low in cortex of brain [6, 7], we can assume that increase of soluble copper ions concentrations could down-regulate the dopamine β-hydroxylase activity in brain cortex and as a result decrease the norepinephrine level and increase the amount of dopamine.

The results of the current study showed that maximum level of dopamine produced after 15 min of copper ion injection and after 1 h its concentration started to decrease. After injection of each dose of copper ion (3, 5, and 10 mg/kg) the same results obtained. This outcome could be due to a sudden increase of copper ion concentration and its competition with sulfhydryl inhibitor that led to blocking dopamine β-hydroxylase enzyme and then inhibitor started to interact with copper ions and again the enzyme is free so, the level of dopamine started to decrease.

Chronic treatment of rats with 1.75 mg/kg copper ion could not significantly affect the dopamine synthesis and this could be due to low amounts of injected copper ions and therefore lack of capability of copper ions to compete with endogenous inhibitors. In addition, body protective mechanisms of rats could also block the entrance and aggregation of copper ions in brain tissues since we did not observe any abnormal behavior from rats after injection except for first minutes of treatment.

Since we showed in this current study the effects of aluminum ions by down-regulating the amount of dopamine synthesis, the involvement of copper ions and increase of dopamine production, therefore the results may help to provide effective knowledge to establish the new therapeutic strategies.

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