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Morphological Changes in the Rat Brain Provoked by Prolonged Lithium Intoxication

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In this study, we report morphological changes in the rat brain following repeated lithium administration. Vacuolization was observed in all studied brain regions of lithium-treated rats. The changes were most pronounced in the cerebral cortex and medulla oblongata. The zones of spongiosis were observed both in the border and deeper layers of the cortex. Less intensive vacuolization was registered in pons and the thalamic region. The vacuolization in the rat cerebellum was restricted to the places of missing Purkinje cells. The normal rat brain aging is shown to cause much less pronounced neurodegeneration than the repeated lithium treatment.

Key words: lithium intoxication, rat brain morphology, neurodegeneration

Introduction

Lithium is a first-line drug for acute and prophylactic treatment of bipolar disorders. It has a narrow therapeutic range requiring frequent monitoring to maintain its levels within the therapeutic window. Clinical neurological associations with lithium overdose have been found and pathologic changes related to lithium toxicity have been observed [10]. Although they are mostly transient and reversible, there is growing evidence that lithium can induce long lasting neurological sequelae [1, 6, 8].

Animal models have been insufficiently employed to study the neurotoxicity of lithium. Neuropathological studies have demonstrated neuronal loss and spongiosis in the rat cerebellum following acute lithium intoxication [3] and these data correlate well with findings in humans. However, experimental data on morphological changes in the rat brain after repeated lithium administration are not currently available.

The aim of the present study is to follow up the morphological changes in the rat brain provoked by prolonged lithium intoxication as compared to the normal brain aging.

Materials and Methods

Adult Wistar rats at the age of seven months received four administrations of lithium chloride with a quarter of the acute dose (250 mg/kg body weight) in the course of eight days (0.2 ml dosing volume in saline, i.p.). Healthy aged rats (eighteen-month old) were injected with the same volume of saline and used as controls.

Different regions of the CNS were studied histologically – cerebral cortex, cerebellum, medulla oblongata, mesencephalon, thalamus and pons, using silver-copper staining for neurodegeneration and luxol fast blue – cresyl violet staining for myelin sheath. The silver impregnation was carried out exactly as described by De Olmos and Ingram [2]. All the sections were studied under Leica DM50008 (New York, USA) microscope.

Results and Discussion

Both the therapeutic and toxic side effects of lithium are manifested mainly in the CNS and hence there is considerable interest in understanding the development of neurotoxicity. The mechanism by which the neurotoxic side effects are generated is not known and it may be related to the regional specificity in lithium's brain distribution.

We have developed an experimental rat model of lithium intoxication based on repeated lithium treatment. The silver impregnation technique allowed precise localization of the morphological changes in the rat CNS as well as their topographic distribution in the brain. Structure alterations in the aged rat brains were used as positive controls of the observed neurodegenerative changes.

Vacuolization of the brain tissue and subsequent formation of the zones of spongiosis was observed in all studied regions following prolonged lithium intoxication (Fig. 1). The major histopathologic changes were demonstrated in the cerebral cortex (Fig. 1 A). A dense net of spongiosis vesicles was seen both in outer and deeper cortical layers. The diameter of the vesicles varied from 5 μm to 50 μm. These changes were also well demonstrated by luxol fast blue – cresyl violet staining (Fig.2 A). On the other hand, in the aged animals' cortex only single spongiosis vesicles of smaller size were found mostly in the outer cortex (Fig. 3 A). Intensive compact areas with spongiform changes were also found in medulla oblongata (Fig. 1 C). Their distribution was homogenous and the vesicles were smaller that those in the cortex. Less pronounced vacuolization was registered in pons (Fig. 1 F) and the thalamic region (Fig. 1 E). The cerebellum (Fig. 1 B) and mesencephalon (Fig. 1 C) were the least affected by the intoxication. Purkinje cell loss was noted in the border zone between molecular and granular layers (Fig. 1 B, Fig. 2 B), but the classical picture of spongiosis was missing. The aged rats' cerebellar cortex revealed only small spongiform vesicles distributed inconsistently in the molecular and granular layers but no loss of Purkinje cells was observed (Fig. 3 B). The silver impregnation technique proved to be more convenient for the visualization of pathological changes than the classical methods of histology. The morphological changes were also well documented with the luxol fast blue – cresyl violet staining (Fig. 2 A, B).

Our findings differ substantially from the literature data about brain morphological changes after lithium treatment. We have demonstrated that the rat cerebellum is the least affected by repeated lithium administration. These results are similar to our previous observations in a mouse model of acute lithium intoxication [9]. In contrast, acute lithium intoxication has been reported to cause widespread vacuolization in the rat cerebellar white matter with no loss of Purkinje cells [3]. Moreover, neuropathological findings in patients have shown that a permanent cerebellar syndrome is the most frequent

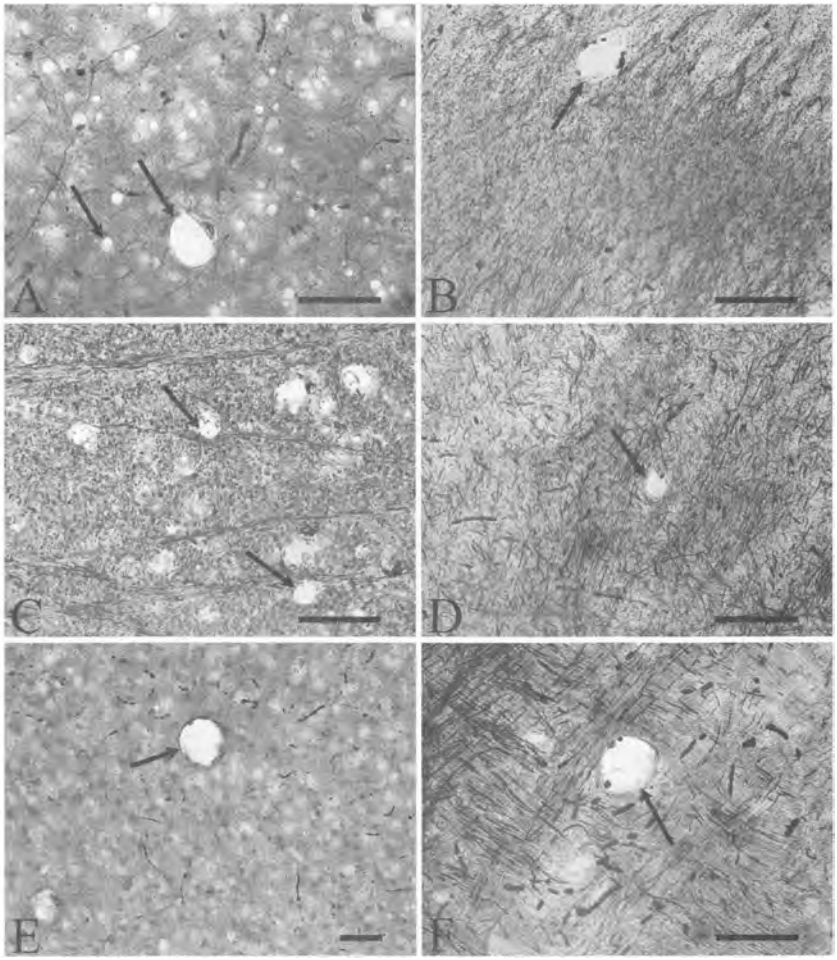

Fig.1. Visualization of neurodegenerative changes in different parts of the adult rat brain after prolonged lithium intoxication using silver-copper staining. A – cerebral cortex – dense net of spongiform vesicles (arrows); B – cerebellar cortex – empty place of a Purkinje cell (arrow); C – medulla oblongata – numeral spongiosis vesicles in the cerebral nuclei of the hypoglossal nerve (arrows); D – mesencephalon – single vesicles of small size (arrow); E – thalamic region – spongiosis vesicles of different size (arrow); F – pons – a single vesicle between the nerve bundles (arrow). Bars = 50 μm.

clinical feature in acute lithium intoxication [7]. These differences could be explained by the dose dependent effects, the mode of application of the drug, different animal strain, etc. The mechanism of these selective pathologic changes is not well understood. Most probably lithium induces metabolic disturbances affecting the cerebellum. Lithium is suggested to act both directly and indirectly on Purkinje cell calcium homeostasis, resulting in excitotoxic effects [5]. It has been speculated to synergize with cytokines and neuroleptics and thereby disrupt calcium homeostasis within Purkinje cells [6]. Lithium is also suggested to influence electrolyte balance, neurotransmitter systems, and carbohydrate metabolism, and may play a role in membrane stabilization [4].

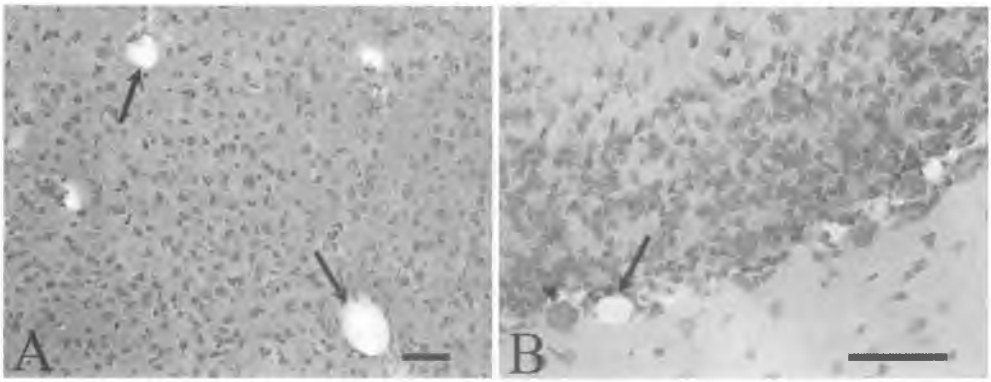

Fig.2. Visualization of neurodegenerative changes in the cerebral cortex (A) and cerebellar cortex (B) of the adult rat after prolonged lithium intoxication using luxol fast blue – cresyl violet staining. Spongiosis vesicles of different size (arrows). A preserved Purkinje cell in the border zone between granular and molecular layers of cerebellum (arrowhead). Bars = 50 μm.

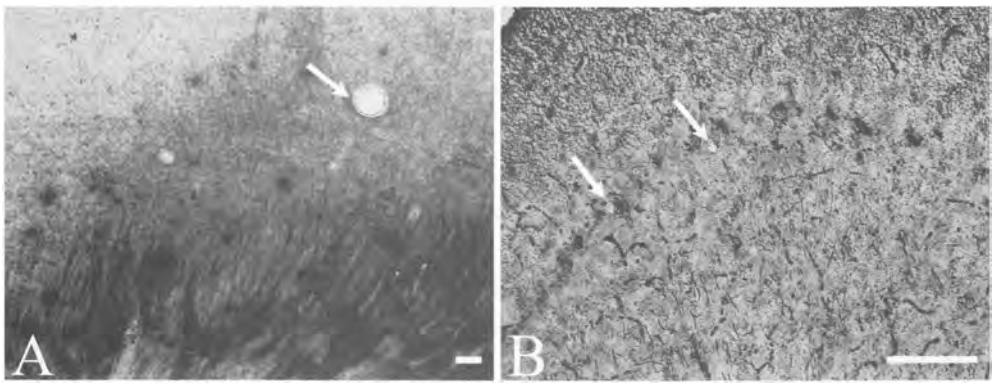

Fig.3. Neurodegenerative changes in aged rat cerebral (A) and cerebellar (B) cortices, visualized by silver-copper staining. Single spongiosis vesicles (arrows), irregularly distributed throughout the white matter. Bars = 100 μm.

In conclusion, prolonged lithium intoxication accelerates neurodegenerative changes concomitant with the normal brain aging. The zones of spongiosis are irregularly distributed throughout different brain regions. The reversibility as well as the quantitative evaluation of the observed changes remains an open question for further studies employing this model.

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