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Original Article

Chronic effects of anti-Alzheimer's drug, Galantamine hydrobromide on cholinergic system of mice brain

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ARTICLE INFO

Article history:
Received 22 December 2012
Accepted 11 June 2013
Available online 27 July 2013

Keywords:
Anti-Alzheimer's drug (GHB)
Behavioural aspects
Cholinergic system
Morphometric aspects

ABSTRACT

Objectives: The present study emphasizes the prolonged effects of an anti-Alzheimer's drug, Galantamine hydrobromide (GHB) on morphometric, behavioural and cholinergic system in mice in the absence of the disease, AD.

Methods: One month old male albino mice, ***Mus musculus*** (20 ± 2 g) were selected as experimental model and GHB as the test drug. The ED_{50} dose (5 mg/kg body weight) was given to experimental mice once in a day up to 180 days continuously.

Results: Observations on the morphometric aspects such as weight, size and also changes in the behaviour pattern of both control and experimental mice were recorded with help of the Morris water maze technique. Various constituents of the cholinergic system viz. acetylcholine content and acetylcholinesterase level were estimated in different regions of brain such as Olfactory Lobe, Hippocampus, Cerebral Cortex, Cerebellum, Pons-medulla and Spinal cord on selected days during the entire treatment schedule lasting for 180 days through standard biochemical assay techniques. From the results, it was evident that GHB exerted severe perturbations in the cholinergic system in all regions of brain on chronic exposure, thus eventually leading to behavioural changes.

Conclusions: From this, it was concluded that GHB, even though exerted positive effects on all the above mentioned parameters which were of course short-lived and during later stages, GHB exerted ill effects. In view of this, particularly, children are cautioned not to consume indiscriminately any kind of memory enhancing drugs or any formulated health drinks containing these chemicals either directly or indirectly for improvement of their cognitive skills.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia is incurable, degenerative and terminal disease first described by German Neuropathologist, Alois Alzheimer in 1906 and was named after him.[1] This disorder usually appears in people older than age 65, but less common forms of the disease appear much early in adulthood.[2,3] AD is characterized by a marked

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0974-6943/$ – see front matter
http://dx.doi.org/10.1016/j.jopr.2013.06.010

loss of cholinergic neurons involved in regulation of learning and memory due to formation of senile plaques and nerofibrillary tangles (NFTs) which are extra cellular deposits of filamentous β-amyloid, a product of amyloid precursor protein. Apart from this, neurons and synapses in the cerebral cortex, subcortical regions, temporal lobe, parietal lobe, parts of the frontal cortex and singulate gyrus have been atrophied which eventually resulted in manifestation of AD.[4] Now-a-days, it has been observed that many of the memory boosters such as Brain Speed Shake, Brain Speed Smoothie, Mocha Focus Delight etc., have chemical substances mimicking the memory enhancing drug, for example GHB.

Apart from these, Nootropics, also referred as smart drugs, memory enhancers, and cognitive enhancers, are drugs, supplements, nutraceuticals, and functional foods which improve mental functions such as cognition, memory, intelligence, motivation, attention and concentration.[5,6] Nootropics are thought to work by altering the availability of the brain's supply of neurochemicals such as neurotransmitters, enzymes, and hormones, by improving the brain's oxygen supply or by stimulating nerve growth. So, these nootropics are now-a-days preferred to be consumed along with memory drinks and food items or sometimes directly. They are also misused by shift workers in companies, industries etc. to reset the body's biological clock in order to lessen the risk of on-the-job injuries caused by impaired alertness.

Currently, among several drugs available for treatment of AD, GHB is one of the latest drug recommended to improve the cognitive functions, and subsequently to treat Alzheimer's patients.[7] In view of this, in the present investigation, it is proposed to assess the long-term effects of memory enhancing drug, GHB on the morphometric aspects, behaviour aspects and cholinergic system of male albino mice in the absence of AD.

2. Materials and methods

One month old male albino mice, *Mus musculus* (20 ± 2 g) were selected as experimental model and an anti-Alzheimer's drug, GHB, as the test drug. Mice were purchased from Indian Institute of Science (IISc), Bangalore and were maintained in the laboratory conditions according to the instructions given by Behringer (1973),[8] 15 days prior to experimentation. The experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals, Government of India (CPCSEA, 2003) and approved by the Institutional Animal Ethical Committee (No.: 05/(i)/a/CPCSCA/IAEC/SVU/KY/BNK/Dt. 22.09.2007).

2.1. Route of administration of the drug

The ED_{50} for GHB to mice was determined as 5 mg/kg body weight.[9] This Effective dose was dissolved in saline and given to experimental mice orally for 180 days continuously. Control groups were maintained for the corresponding experimental groups separately. The following parameters have been studied in both control and experimental groups of mice on selected days namely 30th, 60th, 90th, 120th, 150th and 180th day of chronic exposure.

2.2. Morphometric aspects

The basic morphometric aspects such as size and total body weight of control and experimental mice treated with GHB have been recorded once in five days from 5th day up to 180 days. The data thus obtained was analysed and used to correlate the morphometric changes with the behavioural and biochemical aspects.

2.3. Behavioural aspects

The impact of GHB on the behavioural aspects was assessed with help of the water maze[10] technique. Prior to experimentation, the mice were acclimatized to the maze environment. The animals were divided into 12 batches, each batch consisting of 6 animals. Among them, 6 batches were labelled as control and remaining 6 batches as experimental. The water maze experiment was conducted for both control and experimental animals on the above mentioned selected days, for all six animals in every group separately and the time taken by the individual mice to reach the hidden platform was noted down and the average time was calculated. On comparison between the control and the experimental mice, the performance skills and also the extent of the impact of GHB on the overall behavioural pattern of mice was finally determined.

2.4. Cholinergic system

Acetylcholine content was estimated by the method of Metcalf (1957)[11] as given by Augustinsson (1957).[12] Acetylcholinesterase activity was estimated by the method of Ellman et al, (1961).[13] This method will be consider as a novel method have been adopted for this study.[13]

2.5. Statistical analyses

Data was expressed as mean ± standard error of mean (SEM). Results were statistically analysed by student's *t*-test.[14] The level of significance was at $p < 0.05$.

3. Results

3.1. Morphometric aspects

Changes in general growth parameters such as size and weight of control and experimental mice recorded at selected time intervals revealed that the experimental mice recorded a gradual, continuous and phenomenal gain in their size and body weight during chronic exposure to GHB against their corresponding controls throughout the tenure of the experiment. Maximum weight (22.15%) was gained on 150th day. After 150th day, the experimental mice started losing their body weights gradually up to 180 days (Fig. 1).