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Original article

Inhibitor of Phosphodiestearse-4 improves memory deficits, oxidative stress, neuroinflammation and neuropathological alterations in mouse models of dementia of Alzheimer's Type

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ABSTRACT

The study investigates the potential of Rolipram a phosphodiesterase-4 inhibitor in cognitive deficits induced by streptozotocin (STZ, 3 mg/kg intracerebroventricularly) and natural ageing in mice. Morris water maze (MWM) test was employed to evaluate learning and memory of the animals. Extent of oxidative stress was measured by estimating the levels of brain glutathione (GSH) and thiobarbituric acid reactive species (TBARS). Brain acetylcholinestrase (AChE) activity was also estimated. The brain activity of myeloperoxidase (MPO) was measured as a marker of inflammation. STZ and ageing results in marked decline in MWM performance of the animals, reflecting impairment of learning and memory. STZ treated mice and aged mice exhibited a marked accentuation of AChE activity, TBARS and MPO activity along with fall in GSH level. Further the stained micrographs of STZ treated mice and aged mice indicate pathological changes, severe neutrophilic infiltration and amyloid deposition. Rolipram treatment significantly attenuated STZ induced and age related memory deficits, biochemical and histopathological alterations. The findings demonstrate the potential of Rolipram in memory dysfunctions which may probably be attributed to its anti-cholinesterase, anti-amyloid, anti-oxidative and anti-inflammatory effects. The study concludes that PDE-4 can be explored as a potential therapeutic target in dementia.

1. Introduction

The transition in world age demography toward older age is associated with an increased risk of neurodegenerative diseases, such as Alzheimer's disease (AD) [1]. Dementia of AD accounts for 50% to 70% of dementia cases [2].

Dementia of AD is characterized by the accumulation of abnormally folded protein fragments i.e. amyloid beta peptide (Aβ) and tau that precipitate in amyloid plaques and neuronal tangles respectively [2,3]. Characteristic feature of AD is progressive loss of memory [4]. Currently only two classes of drugs i.e. acetyl cholinesterase inhibitors like rivastigmine, donepezil etc. and NMDA antagonist mementine are available for clinical use [5]. These drugs have only limited benefits so there is utmost need for a sure-shot remedy which will not only provide symptomatic relief but also halt the progression of disease.

Phosphodiesterases (PDEs) play a vital role in the hydrolysis of cAMP/cGMP. Eleven different families of mammalian PDEs have been identified [6–8]. Both cAMP and cGMP are important second messengers in the mature brain that are directly involved in time-dependent events of memory consolidation [9]. Activation of the cAMP-PKA pathway triggers the activation of transcription factors such as CREB, inducing the gene transcription required to consolidate learning and memory [10,11]. Moreover, recent findings have also linked the cGMP pathway to cognition. Basal levels of cGMP are higher in the newborn brain than in the adult brain, and they decrease with age, a decline thought to be the consequence of increased expression of cGMP-dependent PDEs [12].

PDE4 gene members are distributed throughout the brain and are expressed in various neurons. PDE4 specifically hydrolyzes cAMP to inactive AMP. There are four genes (PDE4A, 4B, 4C, and 4D) that encode over 20 different variants of this enzyme [13]. The differential distribution of the four PDE4 subtypes (PDE4A-D) in the brain [14] reflects their distinct roles in the CNS, which may be attributed to the regulation of cAMP/CREB signaling. Based on its

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predominant expression in hippocampal CA1 [14], PDE4D appears to be the main subtype involved in mediating memory consolidation and LTP [15,16]. PDE4 is a key enzyme responsible for production of cytokines in inflammatory cells. It is an intracellular enzyme that encourages inflammation by degrading levels of cAMP. It maintains immune homeostasis by altering the production of pro- and anti-inflammatory mediators. PDE4 inhibitors are recognized to have procognitive [17], neuroprotective and anti-inflammatory effects [18]. Consequently, PDE4 inhibitors have been investigated as treatments option for different diseases, including CNS disorders such as clinical depression [19], anxiety disorders, schizophrenia [20], Parkinson's disease [21], multiple sclerosis [22], Huntington's disease [23], stroke and inflammatory conditions like asthma and psoriasis [22,24]. Accumulating evidence indicates that the inhibition of PDE activity may be a particularly interesting mechanism for memory enhancement [25,26]. PDE inhibitors present a novel therapeutic approach to arrest cognitive decline [27,28] or to possibly reverse this decline with cognitive enhancement [29,30].

Rolipram a first generation cAMP specific PDE4 inhibitor, found to improve memory deficits in various studies [31]. Rolipram also found to ameliorate working memory in rats [32,33] and reversed recognition memory impairment upon sub-chronic treatment is rats or in CBP mice [34,35].

So, far there is no conclusive evidence regarding the potential of PDE4 inhibitor in dementia of AD type, and there is a need to explore the potential of PDE4 inhibitors in dementia.

Therefore the present study has been undertaken to investigate potential of Rolipram a selective PDE4 inhibitor in mouse model of i.c.v. STZ and ageing induced dementia.

2. Materials and methods

2.1. Experimental animals

Mice of either sex weighing 20–35 g were employed in the present study. They were housed in the departmental animal house and were exposed to 12hr light and dark cycle. The animals were acclimatized to the laboratory conditions before experiments. The experimental protocol was duly approved by the institutional animal ethical committee and care of the animals was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. 107/1999/CPCSEA).

2.2. Drugs and chemicals

All drugs were freshly prepared before use. Streptozotocin (STZ) (Sigma Aldrich Co. Ltd., St. Louis, U.S.A.) was dissolved in freshly prepared artificial cerebrospinal fluid (ACSF). Donepezil (Ranbaxy Laboratories Limited, Railmajra, Nawanshahar, India) was dissolved in distilled water. Rolipram (Genetix Biotech Asia Private Limited, New Delhi, India) was dissolves in 0.5% DMSO solution. All the reagents used in the present study were of analytical grade. Rolipram and Donepezil were administered i.p. and STZ was administered i.c.v. in ACSF.

2.3. Induction of experimental dementia by streptozotocin (STZ)

Experimental dementia was induced in young mice (around 4 month old) by intracerebroventricular (i.c.v.) injection of STZ (3 mg/kg) in two divided doses, on the first day and the third day [2,36]. The control group mice were given i.c.v. injection of artificial cerebrospinal fluid.

2.4. Aged mouse model of dementia

Mice of age above 12–15 months served as natural model of Dementia [37,38].

2.5. Rolipram and donepezil administration

STZ treated mice were administered with two i.p. doses of Rolipram 0.05 mg/kg, 0.1 mg/kg daily for 14 days viz. day 9 to day 22 [10 days + 4 days during Morris water maze (MWM) exposure] by suspending the drug in 0.5% w/v of DMSO, while donepezil 0.5 mg/kg i.p. in saline (0.9% w/v) was administered for 14 days viz. day 9 to day 22 (10 days + 4 days during MWM exposure). The animals were administered the vehicle 0.5% w/v DMSO or distilled water 1 h before the retrieval trial conducted on day 5. In the present study, Donepezil was used as a positive control.

2.6. Morris water maze test (MWM)

MWM was employed to assess learning and memory of the animals [39,40]. MWM procedure was based on the principle where animal was placed in a large pool of water divided into four equal quadrants, as animal dislikes swimming, its tendency to escape was accomplished by finding a hidden escape platform. Each animal was subjected to four consecutive training trials (with an inter trial gap of 5 min) each day for four consecutive days in search for a hidden platform. The day 4 escape latency time (ELT) to locate the hidden platform in water maze was taken as the index of acquisition or learning. On the fifth day, the hidden platform was removed. Each animal was allowed to explore the pool for 120 s. Mean time spent in all the quadrants in search of the hidden platform was noted. The mean time spent by the animal in the target quadrant (TSTQ) was taken as the index of retrieval or memory.

The entire MWM test was video-graphed and path length/distance travelled and swim speed of each animal was measured by using ANY-maze video tracking system (v – 4.99).

2.7. Biochemical estimations

Animals were sacrificed by cervical dislocation at the end of the experiment, brain homogenate were collected and subjected to biochemical estimations. Intact brains from each group were preserved in Bouin's solution for histopathological examinations.

2.7.1. Estimation of brain acetyl cholinesterase (AChE) activity

The whole brain AChE activity was measured by the method of Ellman et al. [41]. Change in absorption per min of the sample was read spectrophotometrically at 420 nm.

2.7.2. Estimation of brain thiobarbituric acid reactive species (TBARS) level

The whole brain TBARS level was measured by the method of Ohokawa et al. [42] to estimate the oxidative stress. The absorbance was read spectrophotometrically at 532 nm.

2.7.3. Estimation of brain reduced glutathione (GSH) level

The whole brain GSH level was measured by the method of Boyne and Ellman [43] to quantitate the oxidative stress. The absorbance was read spectrophotometrically at 412 nm.

2.7.4. Estimation of brain nitrite concentration

The brain nitrite level was measured by the method of Sastry et al. [44]. The absorbance was read spectrophotometrically at 545 nm.