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Cholinergic receptor blockade by scopolamine and mecamylamine exacerbates global cerebral ischemia induced memory dysfunction in C57BL/6J mice

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ABSTRACT

Global cerebral ischemia/reperfusion (GCI/R) injury encompasses complex pathophysiological sequalae, inducing loss of hippocampal neurons and behavioural deficits. Progressive neuronal death and memory dysfunctions culminate from several different mechanisms like oxidative stress, excitotoxicity, neuroinflammation and cholinergic hypofunction. Experimental evidences point to the beneficial effects of cholinomimetic agents such as rivastigmine and galantamine in improving memory outcomes following GCI/R injury. However, the direct implications of muscarinic and nicotinic receptor blockade during global cerebral ischemia/reperfusion injury have not been investigated. Therefore, we evaluated the relative involvement of muscarinic and nicotinic receptors in spatial/associative memory functions and neuronal damage during global cerebral ischemia reperfusion injury. The outcomes of present study support the idea that preservation of both muscarinic and nicotinic receptor functions is essential to alleviate hippocampal neuronal death in CA1 region following global cerebral ischemia/reperfusion injury.

1. Introduction

Transient interruption of blood supply to the brain instigates global cerebral ischemia (GCI), which results in neuronal injury in selectively vulnerable cortex, striatum and hippocampus regions [1,2]. The cholinergic projections rich in hippocampus are primarily responsible for learning and memory functions [3,4]. The neuronal damage is more pronounced in hippocampal CA1 region and occurs in a delayed fashion [5–7]. It has been documented that the extent of ischemic damage, survival outcome and long term neurological consequences are majorly attributed to the reperfusion phenomena [8,9]. Although reperfusion following an ischemic insult restores the oxygen and glucose supply to brain, it precedes detrimental neurobiological mechanisms including inflammation and oxidative stress contributing to relentless worsening of cholinergic functions thereby exacerbating neuronal injury and impairment of memory performances [10,11]. These events thus induce loss of hippocampal neurons, memory deficits and cholinergic hypofunction [2,12].

Central cholinergic system is known to play an active role in learning and memory functions as well as a potent regulator of inflammatory immune responses [13–15]. GCI/R injury is characterized by substantial loss of cholinergic neurons, impairment of spatial memory performances associated with decreased brain ACh levels and progressive decline of cholinergic functions in humans as well as experimental animals [16,17]. It has been documented that cholinomimmetic drugs enhance acetylcholine levels in the synapse and modulate cognitive functions [10].

Acetylcholine modulates diverse brain functions through muscarinic and nicotinic receptors. Muscarinic acetylcholine receptors (mAChR), widely expressed in hippocampus and cortex [18–20], play a central role in learning and memory. Blockade of muscarinic neurotransmission by scopolamine, a highly selective muscarinic receptor antagonist impairs spatial working and reference memory performances in rodent models [14,21,22]. Scopolamine is considered as "gold standard" for experimental amnesia extensively used for preclinical testing of new substances designed to treat cognitive impairment [23–27]. Recently many studies reported that scopolamine administration alters the brains redox status leading to memory dysfunction [28–30].

Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels expressed widely in cortex and hippocampus. Nicotine, the prototype nicotinic acetylcholine receptor (nAChR) agonist has been found to improve learning and memory performances through enhanced cholinergic neurotransmission [31,32]. Mecamylamine, a non selective and non competitive antagonist of

Abbreviations: GCI/R, Global cerebral ischemia/reperfusion injury; ACh, Acetylcholine; mAChR, Muscarinic acetylcholine receptor; nAChR, Nicotinic acetylcholine receptor; AChE, Acetylcholinesterase; ChAT, Choline acetyl transferases; CA1, Cornu Ammonis area 1; BCCAo, Bilateral common carotid artery occlusion; LDH, Lactate dehydrogenase; MDA, Malondialdehyde; MWM, Morris water maze.

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nicotinic acetylcholine receptor shows maximum inhibition for α3β4 nAChR [33]. Pharmacological blockade of nicotinic receptors by mecamylamine has been found to decrease ACh synthesis and release from central cholinergic neurons resulting in significantly impaired learning and memory performances [32,34,35]. GCI/R induced dysregulation of central cholinergic signalling, neuroinflammation and neuronal damage have been effectively mitigated by post ischemia treatment with a nAChR agonist [10]. Activation of α7nAChR exert anti-inflammatory effects and ameliorates inflammation in a variety of *in vivo* disease models, including sepsis, arthritis, and myocardial ischemia [36–38]. Exogenous treatment with ACh agonists reduces experimentally induced inflammation suggesting that a decline in cholinergic neurotransmission following neuronal stress probably increases neuroinflammatory response [38].

However, no studies to date have investigated the effect of relative blockade of cholinergic muscarinic and nicotinic receptors on behavioural outcome, oxidative stress and memory dysfunction following GCI/R injury. In the present study, the specific functional effect of sustained muscarinic and nicotinic receptor inhibition on global cerebral ischemia reperfusion injury was assessed by administering scopolamine and mecamylamine for 7 days following BCCAo. Upon completion of treatment, animals were subjected to various behavioural paradigms to assess the spatial working memory and associative memory performances using Morris water maze and passive avoidance tasks. Further, effects of neurochemical alterations like AChE, ChAT and oxidative/ nitrative stress were investigated.

2. Materials and methods

2.1. Experimental animals

Male C57Bl/6J mice (6–8 weeks, 28–30 g) were procured from in-house animal facility and maintained at 23 ± 2 °C, 55 ± 10% humidity under 12 h light/dark cycle with free access to food and water. All experimental procedures were performed between 9.00 am and 6.00 pm under identical conditions and experimental protocols were approved by the Institutional animal ethics committee (IAEC) of Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi. The animal house of the Institute is registered for breeding and experiments on animals with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Welfare Division, Government of India. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Experimental groups

Animals were randomly divided into four experimental groups ($n = 6–8$ per group): Sham operated, Vehicle treated GCI/R (GCI/R), Scopolamine treated GCI/R (Scop GCI/R) and Mecamylamine treated GCI/R (Meca GCI/R). Sham operated group was maintained to evaluate any surgery induced alterations of functional changes.

2.3. Induction of global cerebral ischemia

Bilateral common carotid artery occlusion (BCCAo) was performed to induce global cerebral ischemia according to previously described method [39]. Briefly, an anterior midline incision was made in the neck region under ketamine (100 mg/kg *i.p.*) and xylazine (5 mg/kg *i.p.*) anaesthesia. Both common carotid arteries were carefully exposed and separated from vagus nerve to avoid vagal stimulation. The animals were subjected to surgical induction of global cerebral ischemia by blocking common carotid arteries bilaterally with microclips for a period of 45 min on a temperature controlled surgical platform (to prevent hypothermia) and reperfusion was allowed for a period of 8 days. The sham operated animals underwent the same surgical intervention as ischemic mice, except the occlusion of common carotid arteries. The surgical wounds were locally treated with 2% xylocaine (AstraZeneca, Bangalore, India) and povidone-iodine solution IP (Wockhardt, Mumbai, India). Post ischemic surgical care was taken and the animals were allowed to recover in their home cages for 24 h in a temperature controlled chamber and then returned to the animal quarters. Drug/saline was administered daily to the animals 24 h post-surgery. Seven days post-surgery the animals from all the groups were sacrificed and transcardially perfused with 1X PBS (pH 7.4) followed by 4% paraformaldehyde. The brains were removed and stored at –80 °C until further used (Fig. 1).

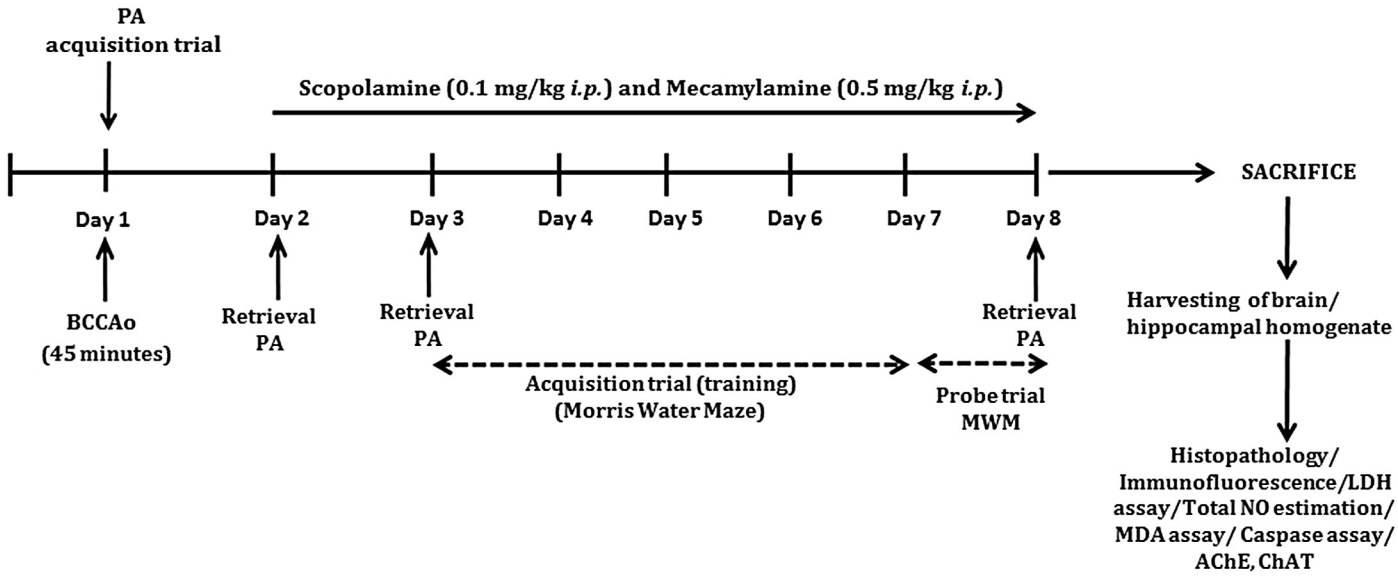

Fig. 1. Experimental design.