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Impact of epigallo catechin-3-gallate on acetylcholine-acetylcholine esterase cycle in aged rat brain

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ABSTRACT

Neurotransmission plays an important role in communication of messages in brain. Cholinergic alterations during aging are associated with learning and memory. Neurotransmitters and enzymes that influence these neurotransmitters are significant in age-associated memory. Neurotransmitters like acetylcholine, serotonin and dopamine levels were studied. Kinetics of acetylcholine esterase was studied. There was an alteration in km and Vm values which was brought back to near-normalcy by EGCG. Behavioural changes were assessed by radial maze experiment. EGCG, a good neuroprotective drug proved to alleviate the behavioural alterations in aged rat brain. Acetylcholine esterase was partially purified from rat brain and assayed *in vitro*. Several modifiers like EGCG and donepezil were added *in silico* and the activity of the enzyme was calculated. EGCG increased the activity when compared to negative control, donepezil. Using bioinformatics tools EGCG, acetylcholine and donepezil were docked with acetylcholine esterase. EGCG formed a good docking-complex with the enzyme. Thus, it shall be hypothesized that the neuroprotective activity of EGCG might be due to its influence on cholinergic neurotransmission thereby improving the cognitive functions of the brain.

1. Introduction

Aging is associated with increased susceptibility to neuronal loss and disruption of cerebral function, either as a component of senescence, or as a consequence of neurodegenerative diseases or stroke. Complete ruling out of the degeneration that occurs during aging is impossible, whereas agents that are capable of neuromodulation will definitely have an impact on memory and cognitive functions and can protect further deterioration. Neurotransmitters are the biochemical interface between the neurons of the central nervous system and consequently have a principal role in the communication of sensory, motor and integrative neuronal messages. Several neuronal systems such as, dopaminergic, cholinergic and serotonergic undergo alterations during aging. Disruption of the cholinergic innervations during postnatal development results in delayed cortical neuronal development and permanent changes in cortical cytoarchitecture and cognitive function (Betancourt and Carr, 2004). A number of pharmacological and lesion studies in humans and animals show that cholinergic neuromodulation is critical for learning and memory (Turchi et al., 2005). Alterations in cholinergic transmission have been implicated in neurodegenerative disorders like Alzheimer's disease and dementia (Herholz et al., 2005).

Acetylcholine (ACh) is synthesized in pre-synaptic terminals from choline and is required for cholinergic neurotransmission in the central and peripheral nervous systems (Goodman and Soliman, 1991). Acetylcholine esterase (AChE) plays a very important role in the ACh-cycle, including the release of ACh (Kouniniotou-Krontiri and Tsakiris, 1989). Disturbance of cholinergic transmission was found in many clinical and neuropathological studies (Coyle et al., 1983; Herholz et al., 2004). The cholinergic system is strictly dependent on both oxidative metabolism and choline supply (Tucek, 1985). The duration of action of ACh at the synaptic clefts is critically dependent on AChE activity (Cooper et al., 2003). Recent studies have thrown light on impact of dietary supplementation on the cholinergic system, particularly during aging (Willis et al., 2009).

Memory decline during aging has been documented in rodents (Ingram et al., 1981). Similar studies in rodents have shown a consistent decline in cortical ACh synthesis and release during aging (Araujo et al., 1990; Vannucchi et al., 1990). AChE mediated behavioural alterations were shown by Chathu et al. (2008). The function of the cholinergic system is known to change during normal aging and in pathological conditions such as Alzheimer's disease (Barnes et al., 2000). Our study aimed to unwind the role of EGCG in modulating the activity of AChE and neurochemical and behavioural changes associated with aging.

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2. Experimental procedures

2.1. Chemicals and reagents

(−)-Epigallocatechin-3- gallate (EGCG) was procured from Sigma Aldrich, USA. All other chemicals were of analytical grade. All other routine chemicals and solvents were of analytical grade and were obtained from SISCO Research Laboratory, India.

2.2. Animal model

Young (3–4 months old; 150 ± 20 g) and aged (above 24 months; 420 ± 20 g) male albino rats of Wistar strain were procured from The Central Animal House Facility, Dr. A.L.M.P.G.I.B.M.S, University of Madras, Chennai, India. The animals were maintained under standard conditions of humidity, temperature (25 ± 2 °C) and light (12 h light/12 h dark). They were fed with standard rat pelleted diet (M/s Pranav Agro Industries Ltd., India); marketed under the trade name Amrut rat/mice feed and had free access to water. Experimental animals were handled according to the University and Institutional Legislation, regulated by the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

2.3. Experimental protocol

The rats were divided into four groups consisting of six animals each. Young animals served as Group I that received saline (0.89% NaCl) alone orally for 30 days. Aged animals receiving saline alone orally for 30 days served as Group II. Young animals that were administered EGCG (2 mg/kg body weight) dissolved in saline through oral gavage for a period of 30 days served as Group III. Aged animals that were administered with EGCG (2 mg/kg body weight) dissolved in saline through oral gavage for a period of 30 days served as Group IV (Srividhya et al., 2008; Srividhya et al., 2009).

After the 30 days experimental period, animals were killed by decapitation for the assay of AChE activity. Brain tissues were excised immediately and immersed in ice-cold physiological saline. A 2% homogenate was prepared and employed for the assay of AChE. A set of animals were used for behavioural analysis and trained accordingly.

2.4. Assay of acetylcholine, dopamine and serotonin

Brain cortex was excised from all the animals and homogenised in 0.01 M HCl and used for the further assay of neurotransmitters. Estimation of serotonin (Labor Diagnostika Nord GmbH & Co, China), dopamine (Biosource, Belgium) and acetylcholine (Biovision, USA) were done using ELISA kits according to the instructions provided. The final absorbance was measured colorimetrically using an ELISA reader.

2.5. Assay of acetylcholine esterase

The cerebral cortex was dissected out quickly over ice according to the procedure of Glowinski and Iversen (1966) and used immediately. The activity of acetylcholine esterase was assayed by the method of Ellman et al. (1961). ACh is hydrolyzed by AChE to acetic acid and thiocholine. The catalytic activity is measured by following the increase of the yellow anion, 5-thio-2-nitrobenzoate, produced from thiocholine when it reacts with DTNB. A 2% homogenate was prepared with the excised brain cortical tissues. The reaction mixture constituted the following. To 3.0 ml of phosphate buffer (pH 7.5), 0.1 ml of the enzyme (homogenate), 0.1 ml of DTNB (0.01 M) and appropriate volumes of acetylthiocholine iodide (1.25 M) was added and the reaction was followed spectrophotometrically at 412 nm for 10 min. The protein concentrations in all the samples were estimated by the method of Lowry et al. (1951). Kinetic analysis of the enzyme was appreciated by using a program called hyperbolic regression analysis. Several plots were drawn to obtain linearity and Vm and km values were calculated.

2.6. Behavioural studies (Eight arm radial maze experiment)

The eight-arm radial maze (Taepavarapruk and Song, 2010) consisted of an octagonal centre platform (85 cm in diameter, arm-to-arm) connected to eight equally spaced arms, with a food cup at the end of each arm. A metallic post (100 cm in length) was positioned between two of the arms. Each trial consisted of a training phase and a memory test phase, separated by a delay (from 5 min to 50 min). Before the training phase, four arms were randomly baited with food pellets (Amrut rat/mice feed, M/s Pranav Agro Industries Ltd., India). The other arms were blocked. In the training phase, each rat was given 5 min to retrieve the food from the four open arms. During the memory test phase, all arms were opened and rats explored the maze until they had retrieved food located in the four arms that were blocked during training, or until 5 min had elapsed. An arm entry was defined as movement along the arm to the food cup. All the rats were given equal number of trials. The training period consisted of about 28 days. After the completion of the trial period, about 5 trials were given for each animal for memory assessment. The memory performance was carried out for 5 consecutive days. Criterion performance during the memory tests was defined as five or fewer arm entries to locate four food pellets. Thus, the percentage of performance and time taken were tabulated and analyzed statistically.

2.7. Partial purification of AChE from rat brain cortex

AChE was extracted from adult rat brain by a method similar to that developed by Chan et al., 1972. Total membrane-bound AChE was obtained by homogenizing the whole rat brain in appropriate volume of 0.32 M sucrose containing 1 mM EDTA solution at pH 6.9 (Wenthold et al., 1974). The homogenate was centrifuged at 3000 for 5 min. The pellet was discarded and the supernatant was processed using ammonium sulphate to salt-out proteins by centrifugation. The soluble portion was subjected to fractionation between 7% and 50% ammonium sulphate (w/v). The residual particulate enzyme was further extracted with the EDTA-sucrose medium. The 50% ammonium sulphate pellet was resuspended in 30 mM phosphate buffer, pH 8.0 and dialyzed against the same buffer to remove residual ammonium sulphate. Following dialysis, the fraction was further purified using DEAE cellulose column. Elution with 1 M NaCl in Tris–HCl, yielded partially AChE enriched fraction, which was again dialyzed to remove any residual particles and was used for further studies. 10 μg of the isolated fractions were resolved in 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis by the method of Laemmli (1970) to access the purity of the isolated fractions. Assay of partially purified AChE was carried out *in vitro* by the method of Ellman et al. (1961) in the presence of EGCG and Donepezil, a known inhibitor of AChE.

2.8. Molecular docking

An atom-based computational docking is essential for protein–ligand interaction studies (Kuntz et al., 1994). A bioinformatics tool, AUTODOCK 4.0 has been used to dock the ligands such as EGCG, acetylcholine and donepezil with target AChE enzyme. AUTODOCK 4.0 uses grid-based method for the calculation of