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Role of Glutathione monoester on age-related neurochemical alterations in rat brain

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ABSTRACT

It is quite apparent that the incidence of neurodegenerative diseases in both men and women increases in a logarithmic fashion with age and begins to rise much more rapidly after the age of 60. Brain aging is accompanied by structural and functional changes at cellular and tissue levels such as increase in free radical generation, lowered antioxidant defenses, decrease in number of neurons, decrease in the activities of enzymes, (g) decrease in impulse transmission. The present study was aimed to assess the neuromodulatory role of Glutathione monoester (GME) when administered intraperitoneally (12 mg/kg body weight) for 20 days on acetylcholine esterase (AchE) activity, levels of neurotransmitters such as dopamine, serotonin, norepinephrine and rotorod behavioral analysis in discrete brain regions of young and aged male albino Wistar rats. Age-related decrease ($p < 0.05$) in acetylcholine esterase activity, neurotransmitter levels and also decrease in sensorimotor performance was observed. GME administration was effective in restoring these neuronal parameters in aged rat brain regions. Thus GME act as a neuromodulator in discrete brain regions of aged rats.

1. Introduction

Aging is a process that results in the loss of cellular function with resultant increases in mortality and represents the major risk factor for a plethora of age-related diseases. It is commonly suggested that age-related declines in cellular function occur, in part, due to the absence of selective pressures during evolution, since the life-span of individuals within animal populations has been relatively short over the evolutionary time-scale (Kirkwood and Austad, 2000). Loss of neurotransmitters, their receptors, and response to neurotransmitters are key manifestations of neurological aging (Enna et al., 1987). Recent studies have suggested that oxidative damage due to free radicals may be important in several neurological disorders (Choi and Yu, 1995; Knight, 1997). Rapid auto-oxidation of catecholamines generate toxic free radicals, semiquinones, hydrogen peroxide, neuromelanin, which further increases hydroxyl radical (OH) production (Urabe et al., 1994), thereby leading to neuronal loss (Kish et al., 1992). Toxic free radicals affect the sulfhydryl groups present in the carrier molecule and thus affect neurotransmitter transport (Berman et al., 1996).

Numerous neurochemical studies using both animals and humans have revealed age-related changes in the activities of neurotransmitter enzymes and their receptor binding capacity. Age-related decline in cholinergic function is thought to be partially responsible for short-term memory disorders during senescence. The major marker of cholinergic metabolism is the activity in which the hydrolytic enzyme acetylcholine esterase (AChE) allows a precise temporal control of synaptic activation by rapidly hydrolyzing the neurotransmitter acetylcholine (Ach) into acetate and choline (Prall et al., 1998). Neuronal communication mediated by the myriads of synapses is mainly mediated by neurotransmitters, although there are also electrical synapses. Neurotransmitters can be defined as chemicals released from neurons that can act on specific receptors. Among the important neurotransmitters in the brain are dopamine (DA), norepinephrine (NE) and serotonin (5-HT). The amount of each neurotransmitter varies in different regions of the brain and particular subsets of neurons within those regions. Alterations in the levels of individual neurotransmitters or in their synthesis or release can affect synaptic transmission.

Research shows that the hippocampus mediates place learning, whereas the cortex is critical for acquiring the rules that govern performance of particular tasks (i.e., procedural knowledge)

Abbreviations: GME, Glutathione monoester; ROS, Reactive oxygen species; AChE, Acetylcholine esterase.

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(Joseph et al., 2005). It appears that the striatum regulates spatial orientation that involves response and cue learning (Devan et al., 1996) and also substantial research indicates that factors such as oxidative stress (Shukitt-Hale, 1999) may be major contributors to the behavioral decrements seen in aging. In animal models, cognitive function is usually measured in a maze and motor function is measured with a battery of different tests, such as those that assess the time a rodent can remain on an accelerod (a slowly rotating rod).

Impaired antioxidant mechanisms are unable to inactivate free radicals that may induce a number of pathophysiological processes and results in cell injury. The protection rendered by antioxidants in aging and various pathological conditions has been already reviewed by Halliwell, 1992 and is found to be reduced in aging conditions. To protect cells against oxidative damage from oxidants produced during the oxygen metabolism, there must be an effective antioxidant system in the organism out of which, thiol antioxidants may be good for use in neurological disorders. Among the thiols, glutathione (GSH) has got great significance in various disease conditions. GSH is depleted in conditions such as cerebral ischemia-reperfusion, Parkinson's disease and GSH depletion can lead to neurological damage (Packer et al., 1997). Glutathione monoester (GME) contains an ester group, esterified to the glycine of glutathione (GSH). GME is lipophilic, and can enter cells more readily than GSH alone (Anderson et al., 1985), thus providing direct intracellular availability of GSH (Anderson and Meister, 1989). Evidence is growing that GSH plays an important role in the detoxification of ROS in brain (Dringen, 2000). It has been reported that GME can be efficiently transported across the blood brain barrier and split to form GSH intracellularly, when compared to GSH (Martensson et al., 1993). Recent studies by us proved that GME acts as a potent antioxidant by inhibiting lipid peroxidation and revitalizing antioxidants in the brain of aged rats (Murali and Panneerselvam, 2007, 2008). Thus the present study was designed to explore the potential efficacy of GME in modulating the neurotransmitters in the brain of aged rats.

2. Materials and methods

2.1. Source of chemicals

GME was purchased from Sigma chemical company (St. Louis, MO, USA). All other chemicals used were of analytical grade and procured from Sisco Research Laboratories, Mumbai, India.

2.2. Animals

Male albino rats of Wistar strain used in this study were obtained from King's Institute of Preventive Medicine, Chennai, and maintained in a clean rodent room. Animals were housed 2–3 per cage that were fitted with stainless-steel wire mesh bottoms and maintained at a temperature of 28 ± 1 °C under a daily photoperiod of 12 h light/dark cycle. The animals were fed with pellet diet (Hindustan Lever Limited, Mumbai, India) and tap water ad libitum. The commercial rat feed contained 5% fat, 21% protein, 55% nitrogen free extract and 4% fibre (wt/wt) with adequate mineral and vitamin contents. The laboratory animal protocol used for this study was approved by the committee for the purpose of control and supervision on experimental animals (CPCSEA) at University of Madras, Chennai, India.

2.3. Experimental protocol

The animals were divided into four major groups consisting of six animals each, such as

Group I: young control rats (3–4 months old, weighing approximately 130–150 g)
Group II: young rats + GME
Group III: aged control rats (above 24 months old, weighing about 380–400 g)
Group IV: aged rats + GME

GME (12 mg/kg body weight/day) was dissolved in 0.89% physiological saline and administered intraperitoneally for 20 days. Control animals received physiological saline alone. On completion of experimental period, animals were killed by decapitation. Brain was excised immediately; regions were separated according to the method of Glowinski and Iverson, 1966 and immersed in ice-cold physiological saline.

2.3.1. Measurement of acetylcholine esterase activity (AChE)

Acetylcholine esterase activity was determined according to the method of Ellman et al., 1961. 20 mg of brain tissue per ml of phosphate buffer (pH 8.0, 0.1 M) was homogenized in a potter-Elvehjem homogenizer. A 0.4 ml aliquot of brain homogenate was added to a cuvette containing 2.6 ml of phosphate buffer (pH 8.1, 0.1 M). 100 μl of the DTNB reagent was added to the photocell. The absorbance was measured at 412 nm. 20 μl of the acetylthiocholine iodide was added. Changes in absorbance were recorded and the change in absorbance per minute was calculated. The enzyme activity was expressed as micromole of substrate hydrolyzed/g/min.

2.3.2. Histochemistry of acetylcholine esterase

AChE histochemistry was performed as described by Hedreen et al., 1985. Rats were anesthetized with sodium pentobarbital and perfused with 50 mM phosphate-buffered saline (PBS, pH 7.4, 4 °C) followed by 4% paraformaldehyde (4 °C) through cardiac catheter. Brains were removed and post fixed in para formaldehyde for 2 h. This was followed by cryoprotection in 10%, 20% and 30% sucrose gradients. 10 μm thick sections were cut in a freezing monotome. The sections were rinsed in 0.1 M phosphate buffer (pH 6.0) and incubated in the following media (50 ml): 32.5 ml of 0.1 M phosphate buffer pH 6.0; 2.0 ml of 0.1 M sodium citrate; 5 ml of 0.03 M cupric sulfate; 1.0 ml of 5 nM potassium ferricyanide; 25 mg of acetylthiocholine iodide and 9.5 ml of distilled water. The sections were incubated for 30 min at a room temperature and developed in ammonium sulfide solution for 2 min. The sections were dehydrated in ethanol series, cleared in xylene and mounted in permount. Then the sections were viewed in light microscope in 200×. The brown colored end product of the reaction was photographed and the intensity was studied.

2.4. Determination of dopamine, serotonin and norepinephrine

Neurotransmitters dopamine, serotonin and norepinephrine were determined using the previously established method of Jacobowitz and Richardson, 1978. Homogenates of tissues were prepared in butanol: 0.01N HCl (2:1 v/v) and then centrifuged and the supernatant was divided in two parts. To one part of the supernatant, 1.5 ml of 0.01 M phosphate buffer (pH 6.5), was added and vortexed for 20 s. Then centrifuged at 3000 × g and the aqueous layer was taken for the assay. Dopamine and norepinephrine in the phosphate buffer extract were oxidized into fluorophores by adding 0.25 ml of 4% EDTA and vortexed briefly. At 2 min intervals, 0.2 ml of iodine solution and 0.25 ml of alkaline sodium sulphite followed by 0.3 ml of 5N acetic acid were boiled for 5 min and then cooled. Dopamine fluorescence was read after 20 min at excitation 320/emission 385. Norepinephrine fluorescence was read immediately in fluorescence spectrophotometer at excitation 385/emission 485.

To the second part of the supernatant, 5 ml of heptane and 0.5 ml of 0.1N HCl were added and centrifuged. To the aqueous layer 0.2 ml of orthophthalaldehyde followed immediately by 1.5 ml of 10N HCl were added and vortexed. The solution was boiled for 10 min and cooled. Serotonin (5-hydroxy tryptamine) was read at excitation 360/emission 470.

2.4.1. Motor coordination: Rota rod test

Psychomotor coordination was assessed by Rota rod test (Shukitt-Hale et al., 1998). The apparatus consists of a horizontal iron rod 2.5 cm diameter and 15 cm long with roughened surface moving on its axis at a speed of 10 rpm. The animal was kept on a moving rod with its head opposite to the direction of the rod movement. Each animal was given three trials. The time duration for which the animal was able to balance on the revolving rod was noted. Average of the three trials was noted as the motor coordination index. Animals with impaired motor coordination drop off soon. The values are expressed in seconds.

2.5. Statistical analysis

The results are expressed as mean ± standard deviation (S.D.). Differences between groups were assessed by one-way ANOVA using the SPSS software package for Windows (version7.5:SPSS, Chicago, IL). Post hoc testing was performed for inter-group comparisons using the least significance difference (L.S.D.) test; Degrees of freedom are 5, statistical significance at P-values < 0.001, < 0.01, < 0.05 have been given as respective symbols.

3. Results

3.1. Acetylcholine esterase (AChE) activity

AChE has a crucial role in the central nervous system and its implications in the behavioral qualities as well as learning, memory and neurodegenerative diseases have long been known. Table 1 shows the activity of AChE in control and experimental