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Original article

Carnosine reverses the aging-induced down regulation of brain regional serotonergic system

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ABSTRACT

The purpose of the present investigation was to study the role of carnosine, an endogenous dipeptide biomolecule, on brain regional (cerebral cortex, hippocampus, hypothalamus and pons-medulla) serotonergic system during aging. Results showed an aging-induced brain region specific significant (a) increase in Trp (except cerebral cortex) and their 5-HIAA steady state level with an increase in their 5-HIAA accumulation and declination, (b) decrease in their both 5-HT steady state level and 5-HT accumulation (except cerebral cortex). A significant decrease in brain regional 5-HT/Trp ratio (except cerebral cortex) and increase in 5-HIAA/5-HT ratio were also observed during aging. Carnosine at lower dosages (0.5–1.0 μg/Kg/day, i.t. for 21 consecutive days) didn't produce any significant response in any of the brain regions, but higher dosages (2.0–2.5 μg/Kg/day, i.t. for 21 consecutive days) showed a significant response on those aging-induced brain regional serotonergic parameters. The treatment with carnosine (2.0 μg/Kg/day, i.t. for 21 consecutive days), attenuated these brain regional aging-induced serotonergic parameters and restored towards their basal levels that observed in 4 months young control rats. These results suggest that carnosine attenuates and restores the aging-induced brain regional down regulation of serotonergic system towards that observed in young rats' brain regions.

1. Introduction

Aging is a natural biological process during which the accumulation of diverse changes occurs in cells and tissues, limits the adaptive possibilities of an organism, reduces life span, and induces the likelihood of diseases and death (Frolkil, 1982; Harman, 2003; Cefalu, 2011). Several neurological changes during aging at the level of gross structural, cellular, molecular and biochemical aspects of the brain have been reported (Malone and Szoke, 1982; Ueda et al., 1987; Bernerd and Richard, 2000). Aging affects the maintenance of brain function by changing the whole brain activity, including its electrical and chemical activities, which depend on the maintenance of dynamic equilibrium between excitatory and inhibitory processes (Juan et al., 1999). Both the excitatory and inhibitory neurotransmitters of the respective neurons maintained their respective excitatory and inhibitory activities of the brain (Gray and Robinson, 2008). These neurotransmitters (viz. acetylcholine, dopamine, serotonin, GABA, glutamate etc.) changes their steady state levels during aging-induced several neurological diseases including neurodegenerative diseases (Goodman and Gillman, 2001). It is also known that these neurons and their neurotransmitters (e.g., serotonin) not only act individually but also they cross-talk with each other (Mukhopadhayay and Poddar, 1995; Claudio et al., 1996) and affects at the level of both cellular and molecular aspects during aging (Mora, 1999).

Serotonin (5-hydroxytryptamine, 5-HT), an important monoamine neurotransmitter (an metabolic intermediate of tryptophan metabolism; Fig. 1) and precursor of 5-hydroxyindoleacetic acid (5-HIAA), is well linked to mental functions such as aggression, mood, sleep etc (McEntee and Crook, 1991; Saudou et al., 1994; Meltzer et al., 1998; Barry and Casimir, 2000). Frazer and Hansler (1994) have shown that alterations in the brain serotonin concentration can produce several behavioral abnormalities. This 5-HT is oxidized (Paaver et al., 2007) by monoamine oxidase-A (MAO-A), an enzyme (Owen et al., 1977) present in mammalian tissues including brain (Shih et al., 1990). Recently, it has been found that during aging the brain regional MAO-A activity is increased (Banerjee and Poddar, 2015). Moretti et al., (1987) have shown that in different brain regions the 5-HT is apparently decreased with an apparent increased level of its metabolite 5-HIAA though the 5-HIAA/5-HT was significantly higher in aged animals compared to young one. There are many reports in the literature where only

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apparent alteration (both increase and decrease) in brain regional serotonin level with a significant increase in their 5-HIAA level have been observed during aging (Simpkins et al., 1977; Brenan et al., 1981; Ponzio et al., 1982; Bhaskaran and Radha, 1983; Timiras et al., 1983; Marielle et al., 1992). Larer et al. (1995) have shown that the significant reduction in serotonin level in aging may serve as a susceptibility factor in the development of late-life depression.

Like brain serotonin depletion during aging, another endogenous dipeptide (β-Ala-L-His) bimolecule known as carnosine having anti-glycating property (Holiday and McFarland, 2000; Hipkiss, 2009) has been found to be depleted (Margles, 1994; Bellia et al., 2009). Margles (1994) has also shown that the olfactory bulb is an enriched zone of carnosine and smelling sense is lost (hyposmia) with the loss of carnosine concentration in aging-induced diseases. Carnosine is present in muscle, brain and circulation (Kohen et al., 1988; Shelly and Marshall, 1981). It (carnosine) has a calcium sensitizing property (Lamont and Miller, 1992), considered as a mobile organic pH buffer (Boldyrev et al., 1994; Bellia et al., 2011), metal chelator (Kohen et al., 1988; Bellia et al., 2011; Boldyrev et al., 2013), inhibits metastasis (Chuang and Hu, 2008), and has a potential involvement in gene regulation (Quinn et al., 1992). This biomolecule inhibits the 6-hydroxydopamine (6-OHDA)-induced stress in endoplasmic reticulum of SH-SY5Y cells (Yun-Mi et al., 2009). Carnosine reduces the glutamate levels and helps to protect the glutamate transporter-1 (GLT-1) expression in astrocytes exposed to ischemia (Shen et al., 2010). In addition, recently Banerjee and Poddar (2015) have shown that carnosine has an attenuating role on aging-induced increase of brain regional 5-HT catabolizing enzyme, MAO-A activity. These information led authors to understand the status of serotonergic system in brain regions during aging and the effects of carnosine on this system in vivo. The present investigation is aimed to study the role of carnosine on brain regional (cerebral cortex, hippocampus, hypothalamus and pons-medulla) serotonergic system during aging using rats as an experimental model in vivo.

2. Materials and methods

2.1. Materials

5-HT-HCl, L-Trp, 5-HIAA, L-Carnosine, Pargyline-HCl, Probenecid-HCl were purchased from Sigma chemicals (St. Louis, M.O., USA), copper sulfate, sodium-potassium citrate, sodium carbonate, sodium hydroxide, Folin-Cicalteu's phenol reagent, trichloroacetic acid, perchloric acid, citric acid monohydrate, sodium citrate, ethylenediaminetetraacetic acid (EDTA), octane sulphonic acid, glacial acetic acid, tetrahydro furan, HPLC grade methanol, water, acetonitrile were purchased from Merck-India (Worli-Mumbai), India.

2.2. Animals

Male albino rats of Wistar strain were used as the experimental animal. The rats were maintained in a room having a 12 h light/dark cycle and temperature 28 ± 0.5 °C with a constant relative humidity (80 ± 5%). Animals were supplemented with a normal standard laboratory diet and water ad libitum. In the present study the guidelines of the Institutional Animal Ethical Committee (Department of Biochemistry, University of Calcutta, India) were followed and all efforts were made to minimize the number of animals used and their suffering.

2.3. Experimental procedures

The rats were divided into three different main groups (Group I, Group II and Group III) with two sub-groups (sub-group A and sub-group B) each and arranged as group IA and IB; group IIA and IIB; group IIIA and IIIB. Each of these sub-groups contained rats of three different ages (4, 18 and 24 months) and each age group contained 4-6 animals. The rats of sub-group IB, IIB and IIIB were treated intrathecally (i.t.) with carnosine at different dosages of 0.5–2.5 μg/Kg/day, i.t. for 21 consecutive days. The rats of sub-group IA, IIA and IIIA were considered as control groups of corresponding experimental groups (sub-group IB, IIB and IIIB) and were treated with equivalent amount of vehicle (20 μL saline) of carnosine through the same route under similar conditions. The group I was used for the neurobiochemical estimation of the Trp, 5-HT and 5-HIAA steady state level. Group II was used for the estimation of 5-HT accumulation and 5-HIAA declination rate and group III was used for the estimation of 5-HIAA accumulation rate. The animals of both carnosine treated (experimental) and its corresponding control (vehicle treated) groups were sacrificed between 09:00am-10:00am after 4 h of last administration to avoid the circadian effect, if any.

2.4. Collection of brain tissue

The brains of both control and experimental groups were immediately taken out after sacrifice of rats and four different brain regions (cerebral cortex, hippocampus, hypothalamus and pons-medulla) were dissected out following the method as described by Poddar and Dewey (1980). The brain regions were then immediately immersed into liquid nitrogen and used for the assay of the steady state level of Trp, 5-HT and 5-HIAA.

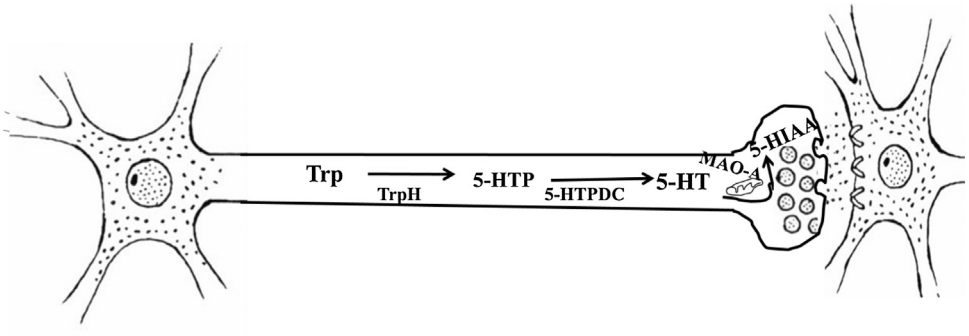

Fig. 1. A diagram of serotonin metabolism in brain.
Trp = Tryptophan, TrpH = Tryptophan hydroxylase, 5-HTP = 5-hydroxytryptophan, 5-HTPDC = 5-hydroxy tryptophan decarboxylase, 5-HT = 5-hydroxytryptamine (serotonin), MAO-A = Monoamineoxidase-A, 5-HIAA = 5-hydroxyindoleacetic acid.