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AGING-INDUCED CHANGES IN BRAIN REGIONAL SEROTONIN RECEPTOR BINDING: EFFECT OF CARNOSINE

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Abstract—Monoamine neurotransmitter, serotonin (5-HT) has its own specific receptors in both pre- and post-synapse. In the present study the role of carnosine on aging-induced changes of [^{3}H]-5-HT receptor binding in different brain regions in a rat model was studied. The results showed that during aging (18 and 24 months) the [^{3}H]-5-HT receptor binding was reduced in hippocampus, hypothalamus and pons-medulla with a decrease in their both B_{max} and K_D but in cerebral cortex the [^{3}H]-5-HT binding was increased with the increase of its only B_{max}. The aging-induced changes in [^{3}H]-5-HT receptor binding with carnosine (2.0 μg/kg/day, intrathecally, for 21 consecutive days) attenuated in (a) 24-month-aged rats irrespective of the brain regions with the attenuation of its B_{max} except hypothalamus where both B_{max} and K_D were significantly attenuated, (b) hippocampus and hypothalamus of 18-month-aged rats with the attenuation of its B_{max}, and restored toward the [^{3}H]-5-HT receptor binding that observed in 4-month-young rats. The decrease in pons-medullary [^{3}H]-5-HT binding including its B_{max} of 18-month-aged rats was promoted with carnosine without any significant change in its cerebral cortex. The [^{3}H]-5-HT receptor binding with the same dosages of carnosine in 4-month-young rats (a) increased in the cerebral cortex and hippocampus with the increase in their only B_{max} whereas (b) decreased in hypothalamus and pons-medulla with a decrease in their both B_{max} and K_D. These results suggest that carnosine treatment may (a) play a preventive role in aging-induced brain region-specific changes in serotonergic activity (b) not be worthy in 4-month-young rats in relation to the brain regional serotonergic activity.
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Key words: aging, brain regions, carnosine, [^{3}H]-5-HT binding.

INTRODUCTION

The neurotransmitters are known to have their own specified receptor in the post synaptic part in which these neurotransmitters bind and generate their specific effect (Boulton et al., 1986). Serotonin (5-hydroxytryptamine, 5-HT) is a well known monoamine neurotransmitter and modulates/regulates different neural activities (Roy and Poddar, 1990; Knobelman et al., 2000; Antonelli et al., 2005; Bonsi et al., 2007; Guiard et al., 2008; Dalal and Poddar, 2009; Dupre et al., 2013) as well as different behaviors (Curzon, 1990; Glennon, 1990; Roy and Poddar, 1990; Dalal and Poddar, 2009; Fidalgo et al., 2013). It is also known that the serotonergic neurons are projected and widely distributed in almost all brain regions in an organized fashion in cortical, limbic, midbrain, hind-brain and brain stem regions (Berger et al., 2009). The 5-HT receptor has been found to be decreased during aging (Heninger et al., 1984; Siever et al., 1984). Frazer and Hensler (1999) have shown that the different types and subtypes of 5-HT receptor oriented knowledge have come out through a very active and interesting way of breakthrough findings. Almost fifteen types of serotonin receptors of seven serotonin receptor families have been discovered based on signaling mechanisms, to understand the mechanisms of serotonergic function (Kroeze et al., 2002).

It is well known that there are different antioxidants which have an influence on different types of 5-HT receptor activity and hence its involvement in different behaviors (Xu et al., 2009, 2010a,b) in mammals during aging-induced stressful conditions. Aging also reduces the activity of different 5-HT receptor subtypes (Yatham et al., 2010; Harvey, 2003; Hasselbalch et al., 2008). Among the 5-HT receptors' family, the 5-HT$_{2A}$ receptor subfamily has been found to be involved in the learning and memory (Harvey, 2003), neuroendocrine function (Van et al., 2001; Zhang et al., 2002) and sleep behavior (Morairty et al., 2008). Therefore, it is not unreasonable to study the influence of an antioxidant, carnosine on 5-HT receptor during aging.

Carnosine is a dipeptide (β-Ala-L-His), endogenous biomolecule (Shelly and Marshall, 1981; Kohen et al., 1988) having antioxidant and antiglycating properties (Holliday and McFarland, 2000; Hipkiss et al., 2001; Bellia et al., 2011). It is present in blood (Shelly and Marshall, 1981), brain and muscle (Kohen et al., 1988). This biomolecule is synthesized by carnosine synthase enzyme in the presence of the rate-limiting amino acid, β-alanine (McCarty and DiNIcolantonio, 2014) and degraded by an enzyme, carnosinase (Joseph and James, 1979; Drozak et al., 2010). It (carnosine) has metal-chelating (Boldyrev et al., 2013) and pH-buffering (Abe, 2000) properties. Carnosine has also a gene

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Abbreviations: i.t., intrathecally; RNS, reactive nitrogen species; ROS, reactive oxygen species.

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regulatory property (Quinn et al., 1992), anti senescence activity (Yuneva et al., 1999) and inhibits the metastasis (Chuang and Hu, 2008). In oxidative and nitrosative-driven neurodiseases carnosine acts as a potent neuro-protectant by scavenging the reactive oxygen species (Bellia et al., 2011; Boldyrev at al., 2013) by modulating the cytoprotective enzymes such as SOD, HSPs and HO-1 (Calabrese et al., 2005; Coban et al., 2013; Davinelli et al., 2013) as well as counteract the metal-induced neurotoxicity (Horning et al., 2000).

Since in recent past study carnosine has been found to attenuate the aging-induced brain regional serotonergic system (Banerjee et al., 2015) and there is no such evidence about the carnosine-mediated changes in serotonin receptor activity, the present study deals with the role of carnosine on brain regional serotonergic activity in the central nervous system.

EXPERIMENTAL PROCEDURES

Materials

5-HT-HCl and Pargyline-HCl were purchased from Sigma Chemicals (St. Louis, M.O., USA). [^{3}H]-5-HT (specific activity 1 Ci/mmol) was purchased from Board of Radiation and Isotope Technology (BRIT, Mumbai, India) and Cocktail 'O' (liquid scintillation solution in toluene) was purchased from Spectrochem Pvt. Ltd. (Mumbai, India). All other chemicals of analytical grade used for the present study, were purchased from Merck-India (Worli-Mumbai), India.

Animals and animal care

The male albino Wistar strain rats were kept in a 12-h dark–12-h light cycle room having temperature 28 ± 0.5 °C with a constant relative humidity (80 ± 5%) for the present study. A normal standard laboratory diet and water *ad libitum* were supplemented to the caged animals. The guidelines of the Institutional Animal Ethics Committee (Department of Biochemistry, University of Calcutta) were followed to minimize the number of animals used as well as their sufferings.

Experimental procedures

The rats of different age groups (4, 18 and 24 months) were divided into three subgroups (subgroup Ia, Ib, Ic; subgroup IIa, IIb, IIc and subgroup IIIa, IIIb, IIIc respectively) and each subgroup was maintained with 4–6 rats. The subgroup Ia,IIa and IIIa was treated with carnosine (2.0 μg/kg/day) intrathecally (i.t.) for 21 consecutive days. The subgroups Ib, IIb, IIIb were considered as control rats of the corresponding subgroups and were treated with same volume (20 μl) of vehicle (saline) of carnosine through the same route for 21 consecutive days. The animals of the subgroup Ic, IIc and IIIc were considered as without vehicle-treated subgroup (Fig. 1). The rats of all the control and experimental groups were sacrificed after 4 h of last administration within 9:00–10:00 h to avoid the circadian effect, if any.

Collection of brain tissue

The brains of both control and experimental rats were collected in a ice-cold (0 °C–4 °C) condition immediately after sacrifice and the four different regions (cerebral cortex, hippocampus, hypothalamus and pons-medulla) were dissected out according to the method described by Poddar and Dewey (1980).

Assay of [^{3}H]-5-HT receptor binding

The [^{3}H]-5-HT binding to its specific receptor in the different brain regions was assayed *in vitro* according to the method described primarily by Bennett and Snyder (1976) and Peroutka and Snyder (1979) and modified by Dalal and Poddar (2009). Briefly, according to the method of Bennet and Snyder (1976), the brain regions individually were homogenized in 20 volumes of ice-cold 0.32 M sucrose solution and the membrane was prepared for the 5-HT receptor binding study. The different brain regional membrane was suspended individually in 50 mM Tris–HCl (pH 7.4) with 10 μM pargyline and 0.1% ascorbic acid and were used immediately for the [^{3}H]-5-HT binding study (Peroutka and Snyder, 1979). The assay mixture containing 0.1 ml [^{3}H]-ligand [final concentration 2.0 nM [^{3}H]-5-HT (Sp. activity 1 Ci/mmol)], 0.1 ml 50 mM Tris–HCl buffer (pH 7.4) (for total binding) or unlabelled 5-HT (10 μM) in the same buffer (for non-specific binding) and 0.8 ml brain regional membrane suspension (containing 0.1 mg protein/ml) was incubated (total incubation volume 1.0 ml) in triplicate at 37 °C for 10 min. For the kinetics study, the [^{3}H]-5-HT (0.01–0.08 nM) was used. After a 10-min incubation, a rapid filter under vacuum condition using Whatman GF/B filter papers was done with 5 ml washing twice using 50 mM Tris–HCl buffer (pH 7.4). The radioactivity of the sample was measured using liquid scintillation β-counter (Perkin Elmer Liquid Scintillation Analyzer, TRI-CARB2800TR) in 5 ml of scintillation fluid (cocktail 'O'). Specific binding was calculated by subtracting the non-specific (in presence of unlabelled 5-HT) binding from total (in absence of unlabelled 5-HT) binding and was expressed as pmol/mg protein. The scatcherd plot was drawn to find out the K_D (nM) and B_{max} (pmol/mg protein) of the [^{3}H]-5-HT binding to its receptor.

Protein estimation

The protein content of different brain regional tissues was spectrophotometrically estimated according to the method of Lowry et al. (1951), using bovine serum albumin (BSA) as a standard.

Statistical analysis

The statistical significance between the results under different conditions was assessed using the analysis of variance (ANOVA) with a post hoc Tukey's test unless otherwise mentioned. $p < 0.05$ was considered as significant.

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