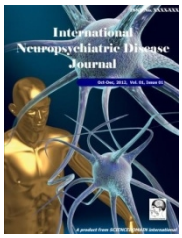

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Striatal Synaptosomal Dopamine and Serotonin Cross-talk Synthesis in Aging Rat

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Authors' contributions

This whole work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: The existence of a dopamine (DA)-serotonin (5-HT) interaction in the brain has been validated by numerous studies. Nevertheless, interaction between DA- 5-HT synthesis in aging brain has not been highly considered. The central aim of this study was to investigate the interaction between DA and 5-HT synthesis in rat brain striatal synaptosomes.
Methodology: Male Wistar rats (3 and 30 months old) were killed by decapitation and the brain sriatal synaptosomes were prepared by discontinuous Ficoll/sucrose gradient technique. DA or 5-HT synthesized during an appropriate incubation period was measured by the synaptosomes in the presence of added substrates (tyrosine or tryptophan) and a monoamine oxidase inhibitor (pargiline).
Results: Dopamine synthesis in the synaptosomes prepared from young animals was markedly inhibited (about 30%) by the addition of 5 μM 5-HT and increasing 5-HT up to 50 μM caused only a relatively small additional inhibition. However, different concentrations of 5-HT (0-50μM) had little effect on dopamine synthesis of the synaptosomes preparations from old animals. In case of 5-HT synthesis, exogenously added 5 μM DA inhibited 5-HT synthesis in the synaptosomes of both ages by about 40%, whereas with higher concentration of DA (10-50 μM) the rate of inhibition was highly pronounced in the old rats as compared to that of young animals.
Conclusion: It is concluded that DA- 5-HT cross reaction might be considered, where

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long-term treatment with L-DOPA of patients suffering from Parkinson's disease renders patients experience variations in response and even psychiatric problems.

Keywords: Aging; dopamine synthesis; serotonin synthesis; synaptosomes; tryptophan hydroxylase; tyrosine hydroxylase.

1. INTRODUCTION

Tyrosine hydroxylase (TH, EC 1.14.16.2) and tryptophan hydroxylase (TPH, 1.14.16.4) are key rate limiting enzymes in the biosynthesis of the neurotransmitters dopamine (DA) and serotonin (5-HT) respectively [1]. A number of investigations on partially purified cell free preparations of TH and TPH have indicated that these enzymes interact with several different molecules, such as non-heme iron(II), molecular oxygen and reduced pterindine cofactor (BH4) to catalyze the conversion of tyrosine to dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5HTP) [2 .3]. Based on kinetic studies of rat TH-catalyzed reaction, a sequential reaction mechanism has been proposed with an ordered binding of BH4 as the first substrate, followed by dioxygen and tyrosine which is subjected to feedback inhibition by catecholamines [4]. This inhibition is suggested to be competitive with BH4 and not with tyrosine [4]. Similar studies on a possible 5-HT/TPH feedback loop have been complicated by the difficulty of preparing an adequate of purified enzyme of sufficient stability [5]. However, a number of reports indicated that both enzymes are tightly regulated by inhibitory feedback via autoreceptors, a phenomenon which has been termed “receptor mediated feedback inhibition” [6,7,8]. These studies suggested that 5-HT and DA inhibit TH and TPH activities respectively. It is now very well known that dysregulation of these enzymes have been implicated in the pathogenesis of Parkinson’s disease and schizophrenia [9,10,11]. Parkinson’s disease is manifested as deterioration of dopaminergic neurons of the substantia nigra, which resulted in a major depletion in DA content of the basal ganglia, whereas, elevated levels of DA, is known to be the etiology of psychosis and schizophrenia. Although numerous pharmacological studies focused on interactions of DA receptors with 5-HT [12,13,14], there is insufficient evidence to point towards the interaction of DA and 5-HT synthesis during aging. Since DA and 5-HT share a common synthetic pathway and the synthesis of both neurotransmitters are influenced by age [15], this study was undertaken to investigate interaction between DA and 5-HT synthesis in the brain striatal synaptosomes of aging rats.

2. MATERIALS AND METHODS

2.1 Animals and Chemicals

Male Wistar rats were housed in a room controlled at 20-24°C and maintained in an alternating 12-h light/dark cycle. Food and water were provided ad libitum. Three months old male rats (young) with weight ranging from 200 to 250 g and 30 months old male rats (old) with weight ranging from 650 to 720 g were used. Animals were maintained with respect to the animal welfare regulation in animal house to the desired age was attained. All chemicals were of reagent grade and obtained from Sigma Chemical Company (Germany). Deionized water was used throughout this study.

2.2 Preparation of Synaptosomes

Three and 30 month old rats were killed by decapitation between 8 to 9 AM and the brain was dissected on ice by the method of Glowinski and Iversen [16]. The synaptosomes were prepared from the dissected stratum essentially as described by Booth and Clark [17]. Briefly the dissected brain striatum from 6 young or 4 old rats were dropped into ice-cold isolation medium (0.32M-sucrose /l mM-potassium EDTA/10 mM-Tris HCl, pH7.4) and chopped into small pieces with scissors. The blood and other debris were washed off and the chopped tissue was then homogenized in a glass Homogenizer using a glass pestle with 0.1 mm clearance. This homogenate was diluted to 60ml with isolation medium and centrifuged at 1200g for 3 min at 4ºC. The supernatant was then centrifuged at 16000g for 10min, producing the crude mitochondrial/synaptosomal pellet. This pellet was resuspended in 30m1 of the Ficoll / sucrose medium [12% (w/w) Ficoll, 0.32M-sucrose, 50M potassium EDTA, pH7.4] and homogenized in the Homogenizer. The suspension was transferred into a centrifuge tube and above this 5 ml of 7.5% Ficoll/sucrose medium [7.5% (w/w) Ficoll, 0.32M-sucrose, 50 pM-potassium EDTA, pH 7.4] was carefully layered. Finally, on top of this 5ml of isolation medium was layered. The tubes were centrifuged at 70000g for 40min at 4ºC. Synaptosomes were gently sucked off from the second interface and resuspended in 10 ml. Cooled incubation medium [125 mM NaCI, 5mM KCI, 1 mM CaCl2, 1 mM MgCl2, 10 mM glucose, 1mM ascorbic acid, and 15 mM sodium phosphate buffer] and centrifuged at 5500g for 10min.The pellet was resuspended in the incubation medium to give a protein concentration of 8 to 10 mg/ml. The optimal pHs for DA [18] and 5-HT synthesis [19] were adjusted by using different concentrations of Na2 HP04 or NaH2P04 at 6.1 and 7.4 respectively. The determination of synaptosomal protein concentration was performed by Lowry's method [20], after lysing the synaptosomal membrane in a 2% (w/v) Na-deoxycholate solution. All processes of preparations were performed at 4ºC.

2.3 Assay of Lactate Dehydrogenase

The integrity of the synaptosomal membrane may be judged by the measurement of lactate dehydrogenase (LDH) activity in the presence and absence of 0.1 % Triton x100 [17]. The assay was performed in the same incubation media with the optimal pHs used for determination of tyrosine hydroxylase (pH6.1) and tryptophan hydroxylase (pH7.4) plus NADH (0.2 mM) at 25ºC using a Pye Unican spectrophotometer (SP 8199). The reaction was started by addition of pyruvate (0.2 mM) and oxidation of NADH (0.2 mM) at 340 nm was monitored against a blank containing all compounds except pyruvate.

2.4 Measurement of DA and 5-HT Synthesis

The method involved determination of the levels of DA or 5-HT by HPLC before and after incubation of synaptosomes, in the presence of pargyline, a monoamine oxidase inhibitor [18]. Briefly, two hundred µl (2 mg protein) aliquots of resuspended synaptosomes were incubated in the presence of either 40 µM tyrosine or tryptophan and 100 µM pargyline. The incubations were carried out at pHs of 6.1 or 7.4 for 15 min at 37ºC and the reactions were stopped by freezing the samples over solid CO2. The concentration of the amines in unincubated samples (blanks) were subtracted from the amine content of the incubated samples to calculate the net rate of DA or 5-HT formed in an appropriate incubation period. For extraction of DA, 200 ml of ice-cold 0.05M perchloric acid and 50 and µl internal standard (2 µl / ml dihydroxybenzylamine, DHBA) were added into the frozen incubated and unincubated synaptosomes and after homogenization DA and DHBA were isolated by

adsorption onto and elution from alumina [18]. Twenty µl of the elute was injected for HPLC determination. For extraction of 5-HT, one ml of ice-cold acidified *n*-butanol was added to the frozen sample and homogenized in an ice bath by an ultraturrax Homogenizer for 30 second. The homogenate was centrifuged at 5000 g. for 10 min and 0.80 ml of the supernatant was added into a vial contained 2 ml heptane, 200 µl 0.1 M perchloric acid and 50 µl internal standard and the mixture was shaken for 15 min. Twenty µl of the aqueous phase was aspirated for HPLC assay [19].

2.5 High Performance Liquid Chromatography

HPLC assays of the amine content of the extracts were carried out on an Altex 15cm X 4.6mm I.D. Ultrasphere-IP column using an Altex model 110A pump and LC-4 amperometric controller with a TL-4 electrochemical detector compartment (Bioanalytical systems). The electrochemical sensor is demonstrated to be highly sensitive and selective for determinations of the amines in the brain, blood and urine [21]. The detector is linked to a Hewlett-Packard Integrator recorder (HP 3380A). The mobile phase for chromatography of DA composed of 90% 0.1M potassium dihydrogen orthophosphate, 0.l mM potassium EDTA, 0.3 mM sodium octyl sulphate and 10% HPLC grade methanol (final pH 3.0). The same mobile phase was used for estimation of 5-HT except that a lower (0.3 mM) concentration of sodium octyl sulphate was present.

2.6 Statistical Analysis

The results are expressed as the amounts of the amine formed/min/mg protein. Results were expressed as mean ± SD. The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Tukey's tests. Statistical significance was considered at $p<0.05$. The statistical analysis was done using the statistical package for the social sciences (SPSS).

3. RESULTS

The LDH latency, given by apparent LDH activity in the presence of Triton x-l00/apparent LDH activity in its absence is well known indicator of the structural integrity of synaptosomes [17]. It was measured after the synaptosomes had been exposed to the incubation medium adjusted to various pHs. The results show that there are no significant differences between LDH latency at pH 6.1 (17.7±0.5, n=6) and at pH 7.4 (17.6±0.6, n=6). However, both synthesis of DA and 5-HT by rat brain striatal synaptosomes are very sensitive to the pH of the incubation medium, in which they are synthesized, but in an inverse relationship, i.e. at acid pH (6.1) DA synthesis is high but that of 5-HT is low, whereas at physiological pH (7.4) opposite is the case. The rate of DA synthesis by the striatal synaptosomal preparations from the young (3 month old) and old (30 month old) animals were 14.6±1.3 and 10.8±07 pmol/min/mg protein respectively. The differences were statistically significant ($P<0.05$). The rate of DA synthesis measured in the synaptosomal preparations obtained from 3 month old animals is consistent with the previous experiments [18]. However, the rate of 5-HT synthesis by the synaptosomes from 3 and 30 month old animals were 2.5±0.3 and 2.6±0.3 pmol/min/mg protein which was not significantly different.

Fig. 1 shows the effect of a range of 5-HT concentrations on DA synthesis by the striatal synaptosomes prepared from 3 and 30 months rats. The results indicate that DA synthesis in the synaptosomes from young animals was markedly inhibited (approximately 30%) by

addition of 5μM of 5-HT (P<0.05) and by increasing 5-HT concentrations up to 50 μM only a relatively small additional inhibition was occurring. However, as can be seen in Fig. 1, addition of different concentrations of 5-HT (0-50 μM) into the incubation mixture had little effect on DA synthesis of the striatal synaptosomes prepared from 30 month old rats.

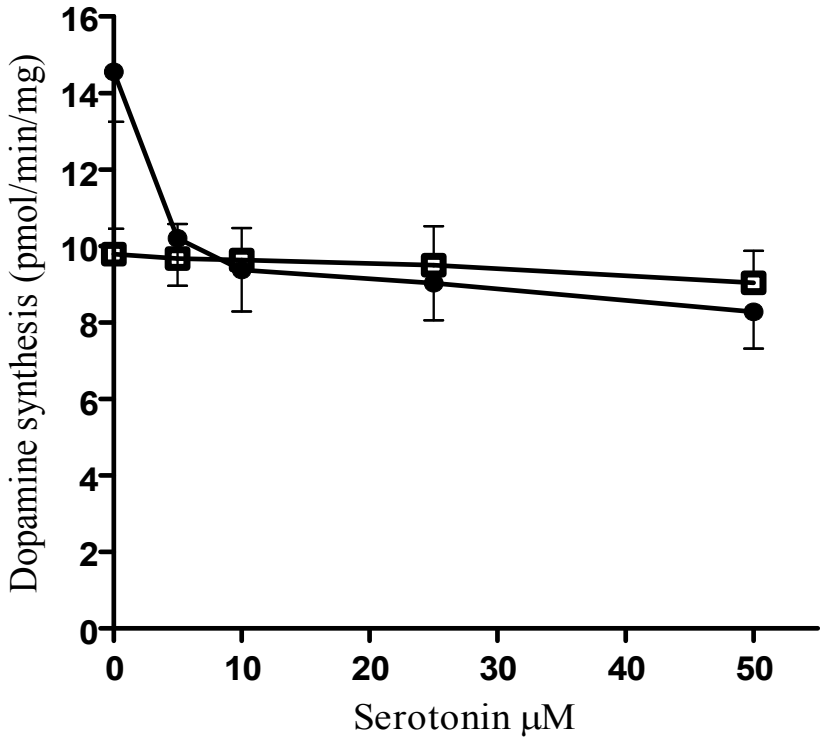

Fig. 1. The effect of different concentration of serotonin on dopamine synthsis in young and old rats. The striatal synaptosomal preparations from 3 months (●-●) and 30 months (■-■) old were included in different concentrations of serotonin (0 to 50 μM), and the rate of dopamine synthsis was measured in the presence of tyrosine (40 μM) and pargyline (100 μM) by HPLC method. Each point represents the mean ± SD of 6 separate experiments performed in triplicate. Differences between young and old rats in the absence of serotonin (0 μM) is statistically significant (P<0.05)

Fig. 2 shows the effect of a range of DA concentrations on the 5-HT synthesis by the strata synaptosomes prepared from 3 and 30 months rats. Exogenously added 5 μM DA inhibited 5-HT synthesis in the synaptosomes of both ages by about 40% (P<0.05), whereas with higher concentration of DA (10 -50 μM), the rate of inhibition was highly pronounced in the synaptosomes of old rats as compared to those of young animals (P<0.05). The rate of inhibition gradually increased with the addition of DA in the synaptosomes of old rats (P<0.05). The dose response curve was a nonlinear fashion for both ages, but the pattern of inhibition was different to that seen for DA synthesis. It is evident from Fig. 2 that the synthesis of 5-HT by synaptosomal preparations from 30 months old rats was more sensitive to high DA concentrations as compared to synaptosomes from 3 months old rats.

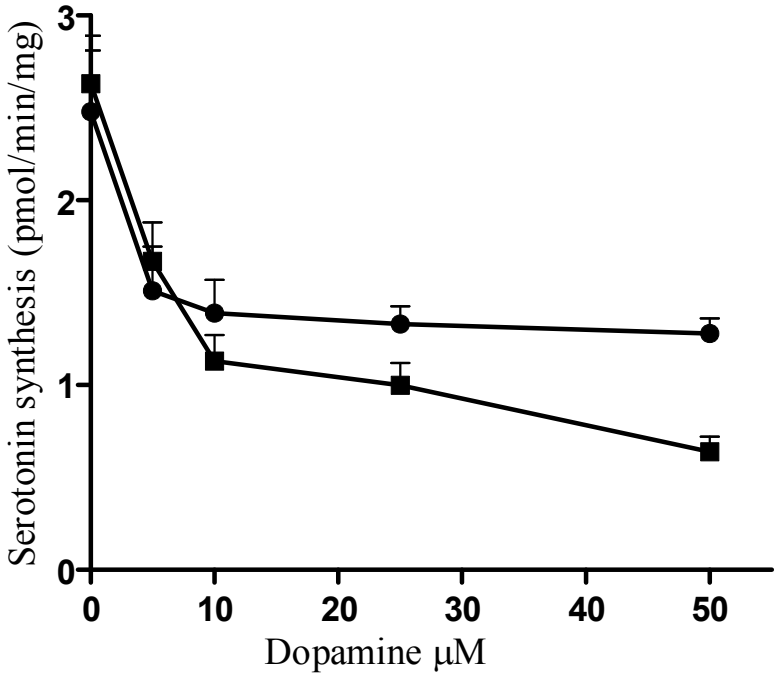

Fig. 2. The effect of different concentration of dopamine on serotonin synthsis in young and old rats. The striatal synaptosomal preparations from 3 months (●-●) and 30 months (■-■) old were included in different concentrations of dopamine (0 to 50 μM), and the rate of serotonin synthesis was measured in the presence of tryptophan (40 μM) and pargyline (100 μM) by HPLC method. Each point represents the mean± SD of 6 separate experiments performed in triplicate. Differences between young and old rats with dopamine concentrations of 25 and 50 μM are statistically significant (P<0.05)

4. DISCUSSION

Membrane integrity of synaptosomes is believed to be an important factor for the maintenance of the enzymes and the reduced pteridine cofactor at near optimal levels of DA and 5-HT synthesis [17,18,19]. As has been indicated previously, the measurement of DA and 5-HT synthesis in the purified synaptosomes in the presence of a monoamine oxidase inhibitor (pargiline) gives rise to the optimal rate at which the neurotransmitters can be synthesized at the synapses from their precursors tyrosine and tryptophan respectively [18,19]. The synaptosomal preparations may have important implications for the final metabolic and membrane integrity of the preparation and hence on its suitability as a model of the nerve ending for studying neurotransmitter interaction in the synaptic region of the neurons of the brain.

The strong and weak responses of the synaptosomes to low (up to 5μM) and high (5-50 μM) concentrations of the exogenously added neurotransmitters Figs. 1 and 2 would be supposed to be affected by at least 2 mechanisms; (a) by uptake of the 5-HT or DA from the

assay mixture thus increasing intra-membrane concentrations of the neurotransmitter and causing inhibition of the TH and (b) by presynaptic receptors, which may influence neurotransmitter release and hence intraterminal neurotransmitters via Ca^{2+} and cyclic AMP dependent mechanisms cause regulation of the enzyme protein itself. This study confirms the hypothesis of an inhibitory control by 5-HT on TH activity in the dopaminergic neurons of the sriatum, indicating that 5-HT could regulate the activity of the enzyme through specific serotoninergic receptor [22,23]. There is strong vidence, indicating that the 5-HT system modulates dopaminergic activity and vice versa. This interaction occurs at the level of the cell bodies in the ventral tegmentum, substantia nigra and medial and dorsal raphe, as well as at various terminal areas of these three nuclei. Multiple types of 5-HT and DA receptors may be involved. To date, the 5-HT1A, 5-HT2A and D2 receptors appear to be the most important for these interactions. Evidence is strong for the model in which 5-HT activity has an overall inhibitory effect upon dopaminergic function, but examples of a facilitatory role exist as well [24]. However, the results of this study indicate that DA synthesis in the synaptosomal preparations of old rat striatum was not affected by the addition of 5-HT. It is suggested that dopaminergic modulatory mechanisms on the presynaptic regions of striatal neurons are not functioning in old rats as they are doing in the neurons of young animals Fig. 1. Conversely, the synthesis of 5-HT in the synaptosomal preparation of old animals was more sensitive to the addition of DA as compared to that of young animals Fig. 2. It appears that increased DA concentrations in the extrasynapic regions of the brain are more critical in aging.

5. CONCLUSION

It is concluded that DA/ 5-HT “cross talk” regulates the neurotransmitter levels at synapses is significantly modulated during the aging process. Such interaction might be noteworthy, where long-term treatment with the DA precursor, L-DOPA of patients suffering from Parkinson's disease [25,26], renders the patients experience fluctuations in response and even psychiatric problems, which might be mediated by decreased 5-HT levels.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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