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A Comparative Analysis of Serotonin Level in Rat Platelets, Serum, and Brain during Aging

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Abstract—Serotonin functions as a neurotransmitter in the central nervous system and takes part in vascular tone, gastrointestinal motility, and blood coagulation at the periphery. New data on a correlation between serotonin level in platelets and cerebrospinal fluid (Audhya et al., 2012) have renewed interest in the hypothesis that considers a platelet as a model of serotoninergic neuron. In this study, using high performance liquid chromatography, we compared the serotonin level in platelets, serum and different brain regions in 6- and 24-month-old rats. It was found that serotonin level decreased from 0.768 to 0.359 μg per 10^9 cells in platelets and increased in midbrain from 0.260 to 0.439 μg per 1 g of wet weight during the animal aging. The differences between young and old animals in the serotonin level in serum and other brain regions were statistically not significant. Hence, despite the attractiveness of the hypothesis considering the platelet as a neuron model the data on the platelet serotonin transport should be extrapolated on the neuronal transport with caution, especially for the aging process.

Keywords: aging, platelets, serotonin, serotoninergic neurons
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INTRODUCTION

Serotonin, or 5-hydroxytryptamin (5-HT), is a biogenic amine affecting numerous functions of the central nervous system. In the central nervous system, serotonin is synthesized and stored mostly in serotoninergic neurons, whereas, at the periphery, the major serotonin producers are enterochromaffin cells of the gastrointestinal tract. Newly synthesized serotonin is stored in vesicles and released into the blood plasma as a response to certain stimuli (Bartrand and Bartrand, 2010). Most serotonin getting into the blood through the intestinal mucosa is rapidly destroyed in liver and lungs (Mohammad-Zadeh et al., 2008). The other serotonin is actively taken up by platelets.

It was supposed that platelets may be an adequate model for serotoninergic neurons (da Prada et al., 1988; Malmgren and Hasselmark, 1988). Both platelets and neurons take up serotonin via a serotonin transporter, transferring it through the cellular membrane (Mercado and Kilic, 2010). Serotonin is stored in dense granules (platelets) or synaptic vesicles (neurons). In human beings, both platelets and serotoninergic neurons contain inactive for serotonin enzyme monoamine oxidase B and do not have serotonin metabolizing monoamine oxidase A (Nagatsu, 2004). Thus, these cells have relatively stable serotonin content. Both cell types (platelets and neurons) serve as basic serotonin storage in organisms.

Using improved methods of serotonin measurement, it has been found that its content in cerebrospinal fluid usually applied for determination of its level in the central nerve system is well correlated with its amount in platelets. The regression coefficient for human and rat cells is 0.97. The high compliance makes possible to replace the invasive method of serotonin determination in the cerebrospinal fluid (as a marker of central system diseases) for the determination of it in platelets, which is a less invasive approach (Audhya et al., 2012).

There are a large number of reports on neuropsychiatric disorders in humans based on serotonin determination in platelets of patient blood (Belendiuk et al., 1980, Antony and Lance, 1989; Coming, 1990; Cook et al., 1993). However, only a few studies have been devoted to physiological parameters that affect serotonin content in platelets. It was reported that serotonin level in platelets of 65-year-old men was lower than in young 18-year-olds. The study was performed on 500 volunteers (Jerney et al., 2000).

The aim of this research was to compare the changes in the serotonin content in blood and central nervous system. Serotonin level was assayed in plate-

[1] *Abbreviations:* 5-HT—serotonin, 5-HTT—serotonin transporter, 5-HTt—gene of serotonin transporter.

Table 1. Age-related serotonin content in rat platelets and blood serum

Object (Experiment number)	Serotonin content in rats of different age (months)	
	6	24
Platelets ($n = 8$)	0.768 (0.144; 2.839)[a]	0.359 (0.039; 0.720)[a]
Blood serum ($n = 6$)	0.792 (0.313; 1.019)	0.456 (0.425; 1.881)

The results are presented as medians (Me) and quartiles (10, 90%); serotonin content is presented in μg/10^9 platelets or μg per 1 mL serum. [a] Difference is statistically significant.

lets, blood serum, and various brain areas of young and old rat males.

MATERIALS AND METHODS

Experiments were done on Wistar rat males at the ages of 6 and 24 months. Seven animals were used in each group. They were kept in a vivarium (12-h light, 12-h dark cycle, standard diet). Rats were decapitated in the second half of the light phase after preliminary narcotization with trichloromethane. Removed brain was placed in a freezer at −85° and stored. Blood collected into two tubes to obtain plasma, and serum was assayed for serotonin content.

Determination of serotonin level in serum and platelets. Blood was collected in tubes with clotting activator, incubated for 10 min at room temperature, and centrifuged at 2000 g for 10 min in a Rotixa 50RS centrifuge. Supernatant was used for the chromatographic assay. To isolate platelets, blood was collected in plastic tubes with EDTA. Samples were centrifuged in a Rotixa 50RS centrifuge at 250 *g* for 7 min at room temperature to obtain platelet-enriched plasma. Two hundred microliters of platelet-enriched plasma was added to 800 μL physiological solution and centrifuged at 4500 *g* for 10 min at 4°C. Supernatant was discarded, and 200 μL bidistilled water was added to the pellet. Platelets were counted with a Sysmex XS-1000i analyzer. The samples were frozen at −20°C to destroy the cells. Thawed samples were centrifuged in a Stat Spin MP multipurpose centrifuge at 12000 *g* for 2 min at room temperature Supernatant was used for further assay.

Determination of serotonin level in homogenates of brain structures. Brain structures were identified with Atlas (Paxinos and Watson, 1982). Homogenates were prepared from forebrain, midbrain, medulla, hypothalamus, tonsils, rhinencephalon and striatum. 10% homogenates from different brain areas were prepared with glass homogenizer in 0.1 N $HClO_4$. Homogenates were centrifuged with a Joan MR23 centrifuge (Thermo Electron corporation, United States) at 14000 *g* for 10 min at 4°C.

Serotonin and tryptophan concentrations were assayed by high performance liquid chromatography with Agilent 1100 equipment (United States) according to the method described in (Koroleva et al., 1996). An Eclipse XDBC18 column (C18 filler, 5-μm granules, column size is 4.6 × 150 mm) was used. The mobile phase was prepared with 0.01 N sodium formate (pH 3.56) and acetonitrile. Solutions were mixed in the increasing gradient from 5 acetonitrile and 95 Na^+ formate parts to reach a ratio of 95 : 5. The flow rate was 0.5 mL/min.

The results were statistically analyzed with Statistica software. Two rat group (old and young) were compared with nonparametric criterion for small samples. The results are presented as medians (Me) and quartiles (10 and 90%).

RESULTS AND DISCUSSION

Serotonin content in platelets and serum of 6- and 24-month-old rats is presented in the Table 1. The comparison of age groups with the Mann–Whitney test showed that serotonin content in platelets of young rats is higher than in old animals ($p = 0.026$). However, in the blood serum, the difference between 6- and 24-month-old animals was not statistically significant (Table 1).

The results obtained with animals confirmed the finding that there is an age-related decrease in serotonin level obtained with a large population of human volunteers (Jerney et al., 2000). Serotonin loss may be caused by age-related changes in the activity of enzymes involved in its synthesis and degradation in peripheral tissues determining its level in blood plasma. Another cause may be changes in the activity of serotonin transporter in the platelet membrane.

Kinetics of serotonin transport in rats genetically selected for extreme values of serotonin content in platelets exhibited the high correlation between serotonin level in platelets and maximal rate of serotonin transport through the platelet membrane (Cicin-Sain et al., 2005). The authors did not find a correlation between serotonin level and Michaelis constant crucial for serotonin affinity to membrane binding sites. Thereby, it is probable that variations of serotonin level in platelets of different age animals are ascertained by the different rates of its uptake by blood plasma cells. The rate depends on the expression of the transporter protein in the platelet membrane.

These observations suggest that low serotonin level in platelets of old rats is a result of declined expression of *5HTt* gene for serotonin protein-transporter. This thought is supported by the findings on age-related changes on binding site number for the platelet serotonin transporter in rats (Slotkin et al., 1997). The authors demonstrated lower number of the transporter

binding sites determined with H^3-labeled paroxetine in old rats (20 months) than in young animals (3 months).

Platelet serotonin transporter is structurally identical to the neuronal serotonin transporter and encoded by the same gene (Lesch et al., 1993). Neuronal serotonin transporter (5-HTT) completes the serotonin functioning as a neurotransmitter with its repeated uptake by presynaptic neuron operating as the major regulator of the serotoninergic synapse. This protein is also a molecular target for currently used antidepressants and narcotic drugs (Lesch, 2001). In addition to completion of the nerve signal transmission by serotonin uptake from the synaptic cleft, 5-HTT helps to refill presynaptic neurons with the neurotransmitter (Torres et al., 2003). *5HTt* gene activity may be modified rapidly (for example, by the protein-transporter phosphorylation) or slowly (for example, at the transcription level). This defines the mechanisms that control synaptic transmission of nerve impulses (Zahniser and Doolen, 2001).

Table 2 shows serotonin content in various brain regions in rat males at the age of 6 and 24 months. Application of the Mann–Whitney test to compare average values of serotonin content (medians) in different regions of male rats revealed significantly increased serotonin level in old rats compared to young animals only in midbrain ($p = 0.026$). A trend toward increased levels of serotonin during aging was observed in the frontal cortex and rhinencephalon. No differences in serotonin level between young and old rats were registered in hypothalamus, tonsils, medulla, and striatum (Table 2).

Table 2. Age-related serotonin content in different brain regions in rats

Brain structure	Serotonin content (μg/1 g tissue wet weight) in rats in age (months)			
	6		24	
	n	Me	*n*	Me
Forebrain	7	0.088 (0.037; 0.128)	6	0.117 (0.053; 0.209)
Medulla	6	0.289 (0.260; 0.360)	7	0.301 (0.256; 0.355)
Thalamencephalon	6	0.274 (0.181; 0.320)	6	0.275 (0.247; 0.364)
Midbrain	6	0.260[a] (0.206; 0.373)	6	0.439[a] (0.268; 0.635)
Hypothalamus	7	0.277 (0.136; 0.389)	7	0.270 (0.168; 0.416)
Tonsils	6	0.142 (0.055; 0.259)	6	0.189 (0.043; 0.377)
Rhinencephalon	6	0.287 (0.178; 0.647)	7	0.420 (0.196; 0.721)
Striatum	7	0.243 (0.121; 0.290)	7	0.243 (0.127; 0.290)

Results are presented as medians (Me) and quartiles (10, 90%); *n*—number of investigated structures. [a] Difference is statistically significant.

Brain structures were also assayed for the content of tryptophan as a precursor of serotonin synthesis. No difference between old and young animals was observed in the amino acid amount in most brain regions. Tryptophan content was higher in rhinencephalon of old rats (7.06 μg/g; 3.60, 9.34) than in young animals (4.45 μg/g; 3.93, 5.42). Correlation analysis revealed a positive correlation between tryptophan content in certain brain regions and serotonin concentration. The correlation coefficient for hypothalamus was 0.59, 0.62 for medulla, and 0.49 and 0.43 for midbrain and striatum, respectively. These findings suggest that age-related difference in serotonin content does not result from altered rate of inflow or synthesis.

The difference in serotonin level in different brain regions may be defined by various expression of serotonin transporter, as it was observed in experiments with platelets. Morphologically and anatomically different dorsal and median raphe nuclei differ in expression of serotonin transporter. Transporter mRNA level varies in rat strains (Zahniser and Doolen, 2001) and after pharmacological manipulation (Kuroda et al., 1994; Fernandez et al., 2003), which correlates to the difference in the number of sites binding 5-HTT defined with H^3-labeled paroxetine (Kuroda et al., 1994; Romero et al., 1998).

The difference between dorsal and median raphe nuclei and their projection areas for the number of binding sites determined with H^3-labeled cytalopram was observed in brain of Wistar-Zagreb and 5-HT rats selected for constructively modified serotonin homeostasis in platelets (high and low) (Romero et al., 1998).

The data on serotonin content in various regions of the central nervous system are scanty. It was reported that, in *5-HTt* knockout mice and rats, the extracellular serotonin level determined with microdialysis was increased in all brain structures (Arora et al., 1993).

There are reports that there is a decreased number of binding sites for serotonin transporter in brain with age (Caspi et al., 2010). Others have shown that the number of transporter binding sites for 5-HTT in rat brain increased with age (Slotkin et al., 1997). These contradictions are attributed to an imperfection of methods and possible species differences in gene expression of the serotonin transporter in experimental animals.

To compare the data on serotonin content in platelets calculated for 10^9 cells it was necessary to prove that there was no change in the platelet volume in old

animals. The average platelet volume estimated with a Sysmex XS-1000i analyzer (see Materials and Methods) was 8.6 fl in young rats and 8.9 fl in old ones (the difference is not statistically significant). Therefore, our experiments demonstrate that age-related changes in serotonin content in platelets and brain are differently directed. Serotonin level was more than twice decreased in platelets of old rats, whereas its concentration in midbrain was increased by 1.7 times.

Expression of the serotonin transporter gene in brain and platelets was different. 5-HTt expression in platelets and midbrain was compared in two sublines of Wistar-Zagreb 5-HT rats with high and low serotonin content in platelets (Caspi et al., 2010). It was found that, unlike in platelets of two sublines, the difference in 5HTt gene expression in midbrain of these animals was not so evident. This shows that serotonin homeostasis in brain is controlled more effectively than at the periphery (Bordukalo-Niksic et al., 2004). Although the concept of "a platelet as a neuron model" seems to be catching on, the results of platelet serotonin transport should be extrapolated carefully onto neuronal transport, especially with increased patient age.

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