Coll. Antropol. **32** (2008) Suppl. 1: 37–41
Original scientific paper

Expression of 5HT-1A and 5HT-1B Receptor Genes in Brains of Wistar-Zagreb 5HT Rats

Tatjana Bordukalo-Nikšić, Gordana Mokrović, Branimir Jernej and Lipa Čičin-Šain

Laboratory of Neurochemistry and Molecular Neurobiology, Department of Molecular Biology, »Ruđer Bošković« Institute, Zagreb, Croatia

A B S T R A C T

By selective breeding, two sublines of rats with high or low activity of platelet serotonin (5HT) transporter (5HTt) have been developed (Wistar-Zagreb 5HT rats). Previous studies demonstrated significant differences between the sublines in the expression of platelet 5HTt at the level of both, mRNA and protein. Pharmacological studies showed marked alterations in brain 5HTt function, indicating differences in central serotonin homeostasis, although analysis of regional brain 5HTt gene expression did not show analogous differences. In this study, we searched for possible changes in the expression of the two central 5HT receptor subtypes: 5HT-1A and 5HT-1B, both participating in the regulation of brain 5HT transmission. Semi-quantitative RT-PCR, with three different housekeeping genes as internal standards, showed no differences in the levels of 5HT-receptor expression between the sublines. Results suggest that constitutional alteration of 5HT homeostasis, induced by selective breeding for the extremes of platelet 5HTt activity, did not cause measurable changes in the expression of central 5HT-1A (hippocampus) and 5HT-1B (striatum) receptors in the mentioned rat sublines under physiological conditions.

***Key words**: serotonin receptor, gene expression, rat brain*

Introduction

Serotonin (5-hydroxytryptamine, 5HT) exerts its effects through at least 15 different receptors, which are grouped into 7 main types. All 5HT receptor subtypes, except for 5HT-3 receptor, are members of the G-protein coupled receptor superfamily[1].

Serotonin-1A (5HT-1A) receptors are the most extensively studied of all 5HT receptor subtypes and have been shown to have a role in physiological functions such as cognition and emotions; they also play an important role in regulation of neuroendocrine functions and responses to stress[2], as well as in neural development[3]. They are involved in etiopathology of certain neuropsychiatric disorders, e.g. anxiety and depression[4,5], and in the mechanisms of action of antidepressant drugs[6].

5HT-1A receptors are expressed both, postsynaptically to the 5HT neurons (in forebrain regions, particularly in hippocampus) and presynaptically, on soma and dendrites of the 5HT neurones (in the raphe nuclei region)[7]. This heterogeneity in distribution of 5HT-1A receptors has been associated with diversity in pharmacology and signal transduction characteristics of the receptor[3,8]. By regulation of neuronal firing, somatodendritic 5HT-1A autoreceptors are particularly important in regulation of serotonergic neurotransmission, as demonstrated in electrophysiological and microdialysis studies[9]. Postsynaptic 5HT-1A receptors seem to participate in modulating release of other neurotransmitters (acetylcholine, noradrenaline)[7], contributing thus to the overall transmission[10]. Mice with genetically inactivated 5HT-1A receptor (5HT-1A knockout mice) develop anxiety-like phenotype and increased responsiveness to stress[11]. Agonists of 5HT-1A receptors act as psychoactive agents with both, anxiolytic and antidepressant effects[2], and, in some paradigms, display anti-aggressive effects[12].

Serotonin-1B receptors (5HT-1B) have been found on both, serotonergic and non-serotonergic neurones, acting as presynaptic auto- and hetero-receptors. By regulating terminal serotonin release[12], they have an important role in controlling synaptic 5HT transmission. They are involved in several physiological functions, behav-

Received for publication June 1, 2007.

iours and diseases, including locomotor activity, drug abuse, migraine, anxiety states and aggressive behavior[12,13]. 5HT-1B knockout mice showed increased aggressiveness, increased exploratory activity[14] and increased vulnerability to cocaine[15]. Similarly to 5HT-1A receptors, expression of 5HT-1B receptors is also influenced by antidepressant drugs[16]. Drugs acting as 5HT-1B agonists reduce aggressive behavior[12,13] and have shown efficacy in the acute treatment of migraine[1,13].

By selective breeding for the extreme values of platelet serotonin level (PSL), we have previously developed two sublines of rats differing markedly in this trait[17]. Further studies indicated platelet serotonin transporter (5HTt) as the protein substrate being under genetic pressure and underlying the observed differences in PSL between sublines[18,19]. This allowed more specific selective breeding for the extreme values of platelet serotonin uptake (PSU) velocity, which finally resulted in development of two rat sublines with high and low 5HTt velocity, referred to as Wistar-Zagreb 5HT rats. It seems that, besides in platelets, selection process affected serotonin homeostasis in general, including the brain[20,21]. Indeed, pharmacological challenge with citalopram (SSRI antidepressant) resulted in markedly different response, with respect to the extracellular 5HT level in ventral hippocampi, between 5HT-sublines[21]. Behavioral studies showed differences in learning ability, motor functions and anxiety-like behaviors[22,23], and there is evidence that these sublines differ in alcohol intake and preference[24]. Detailed characterisation of high-5HT and low-5HT animals at the neurochemical and molecular genetic levels are in course. Although studies on the expression of neuronal 5HTt gene in brain cortex did not show significant differences between the sublines[25], the observed behavioral/functional differences strongly indicate hyper- and hypo-serotonergic neurotransmission in brains of high-5HT and low-5HT subline, respectively, making studies of possible adaptational changes in neuronal 5HT receptors warranted.

This study was aimed at the mRNA encoding the two 5HT receptor subtypes, 5HT-1A and 5HT-1B in hippocampus and striatum, respective regions of their abundant expression.

Materials and Methods

Studies were performed on sublines of Wistar-Zagreb 5HT rats, developed as described previously[25]. Shortly, at the age of 5–6 weeks, PSL and PSU were determined in offspring of each generation and the males and females displaying the extreme values of PSU were mated to start a new generation of the high-5HT and low-5HT sublines, respectively. In this study females from the 15th breeding generation, aged about 12 months were used (N = 8 per group). They were housed three per cage with free access to commercial rat chow and tap water. All experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

PSL and PSU were measured in samples of platelet-rich plasma (PRP) obtained from 1 mL of venous blood, according to reported procedure[26,27]. Tissue (hippocampi and striata) were quickly excised from the fresh brains, after decapitation of the animal, and rapidly frozen in the liquid nitrogen.

RNA was isolated from brain tissue by standard acid guanidinium-phenol-chloroform extraction[28]. Isolated RNA was further purified with RNeasy micro kit (Qiagen, Germany), which included removal of genomic DNA. 1 μg of total RNA was used for synthesis of single-stranded cDNA. Reverse transcription (RT) mixture contained following constituents: 2 μL of oligo $d(T)_{16}$ primers, 1× RT buffer, 1 mM deoxynucleotide triphosphate, 5 mM $MgCl_2$, 20 U of RNasIn and 15 U of AMV reverse transcriptase (Promega, USA).

PCR was performed in a total volume of 20 μL, which contained 1 μL of RT mixture, 1× PCR buffer, 0.2 mM dNTP and 0.6 U Taq DNA polymerase (Applied Biosistems, USA). Final concentrations of $MgCl_2$ and specific oligonucleotide primers, as well as sequences of oligonucleotide primers[25,29,30] and expected RT-PCR product sizes are given in Table 1. All oligonucleotide primers were custom synthesized by Life Technologies (Vienna, Austria). The PCR conditions were as follows: 30'' at 94 °C, 30'' at 60 °C and 45'' at 72 °C. To determine the appropriate number of PCR cycles, at which linear phase is maintained, for each primer set PCRs at different number of

TABLE 1
PCR CONDITIONS AND PRIMER SEQUENCES

target mRNA	$MgCl_2$ concentration (mM)	primer concentration (μM)	optimal number of PCR cycles	primer sequence	expected product size (bp)
5HT-1A	1.0	0.8	28	5' CCCCCCAAGAAGAGCCTGAA 3' 5' GGCAGCCAGCAGAGGATGAA 3'	336
5HT-1B	1.5	0.8	32	5' CTGCTAAAAGAACTCCCAAAA 3' 5' TTGGGTGTCTGTTTCAAAATC 3'	262
GAPDH	2.0	0.4	24	5' AGAACATCATCCCTGCATCC 3' 5' TCCACCACCCTGTTGCTGTA 3'	367
β-actin	2.0	0.4	26	5' GAAACTACCTTCAACTCCATC 3' 5' CTAGAAGCATTTGCGGTGGACGAT 3'	303
cyclophylin B	2.0	0.4	26	5' CCATCGTGTCATCAAGGACTTCAT 3' 5' TTGCCGTCTAGCCAGGAGGTCT 3'	216

cycles, and with given amount of cDNA (1 μL), were run. Optimal number of cycles for each set of primers is also given in Table 1. Three typical housekeeping genes (GAPDH, β-actin, cyclophylin B) were used as internal controls. Expression of 5HT-1A receptor gene was studied in hippocampus, and expression of 5HT-1B receptor gene in striatum.

10 μL of PCR products were separated on a 1.5% agarose gel with TAE buffer, containing 0.1% ethidium bromide. Electrophoresis of PCR products from each reaction revealed single band of the expected product size (see Table 1). Gels were scanned on the Image Master VDS apparatus. Digital images were densitometrically analysed using Image Master™ software (Pharmacia Biotech, USA).

Relative amounts of 5HT-1A and 5HT-1B mRNAs were expressed as ratios between the amount of PCR product of each of the 5HT receptor cDNAs and the amount of the PCR product of each of the control gene cDNAs. Results are given as means±standard deviations (X±SD). Significance of the differences between the mean values was checked using t-test, with the level of significance set at 0.05.

Results

By the use of described protocols relative 5HT-1A and 5HT-1B mRNA abundances were compared in brains of animals from high-5HT and low-5HT sublines of Wistar-Zagreb 5HT rats. The same groups of animals demonstrated approximately twofold difference in their platelet 5HT transporter velocity (high-5HT subline: 0.71±0.18 nmol 5HT/mg protein/min; low-5HT subline: 0.38±0.05 nmol 5HT/mg protein/min).

5HT-receptor mRNA from brains of the two high-5HT and the two low-5HT animals were analysed in the same experiment simultaneously with the three corresponding reference genes (GAPDH, β-actin, cyclophylin B), used as internal standards for normalising 5HT-receptor messages. Data are given in Table 1.

Figure 1 shows the results of 5HT-1A gene expression in hippocampi of Wistar-Zagreb 5HT sublines. 5HT receptor gene/reference gene signal ratios were calculated for all three referent genes and then the level of 5HT-1A expression in low-5HT subline is given as a percentage of the relative 5HT-1A expression in high-5HT subline (designated as 100%). No difference in the expression of 5HT-1A gene in the hippocampus was observed between sublines, regardless of the housekeeping gene used to normalise receptor signal (high-5HT subline: 0.66±0.09, 0.85±0.19, 0.98±0.26; low-5HT subline: 0.74±0.29, 0.88 ±0.13, 0.94±0.14; signal intensities relative to the expression of GAPDH, β-actin, and cyclophylin B, respectively).

Analogous analysis of 5HT-1B gene expression in striata of high- and low-5HT sublines is shown in Figure 2. Again, no differences between the sublines were found (high-5HT subline: 0.44±0.06, 0.62±0.11, 1.22±0.27; low-5HT subline: 0.42±0.09, 0.62±0.17, 1.23±0.39; signal intensities relative to the expression of GAPDH, β-actin, and cyclophylin B, respectively).

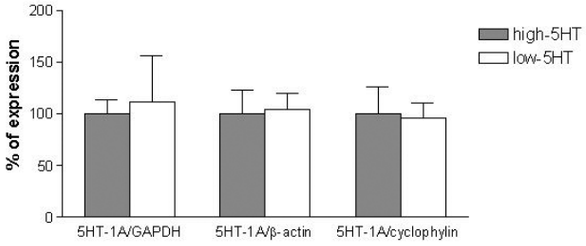

Fig. 1. 5HT-1A expression in hippocampi of animals from high-5HT and low-5HT sublines. Relative 5HT-1A mRNA levels are expressed as ratios with respect to different control genes (GAPDH, β-actin and cyclophylin). The level of 5HT-1A expression in low-5HT subline is given as a percentage of expression in high--5HT subline (=100%). Each column represents X±SD of 8 animals. No significant differences were detected.

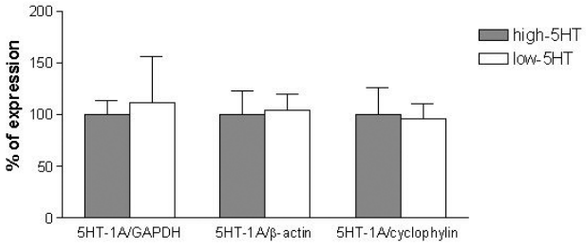

Fig. 2. 5HT-1B expression in striata of animals from high-5HT and low-5HT sublines. Relative 5HT-1B mRNA levels are expressed as ratios with respect to different control genes (GAPDH, β-actin and cyclophylin). The level of 5HT-1B expression in low-5HT subline is given as a percentage of expression in high-5HT subline (=100%). Each column represents X±SD of 8 animals. No significant differences were detected.

Discussion

By using the originally developed method for the *ex vivo* monitoring of PSU kinetics in the individual rat[27], the directed genetical selection of animals for the extreme values of PSU resulted in development of two sublines of »Wistar-Zagreb 5HT rats«[31]. They demonstrated pronounced differences in the velocity of platelet 5HTt, which is encoded by the same gene as its central counterpart. Further neuropharmacological and behavioural studies suggested that the selection process had functional impact also on the brain serotonergic homeostasis[20,21,22,23]. Although molecular genetic studies showed clear differences in platelet 5HTt mRNA and protein levels between the sublines[18,19], studies on the expression of 5HTt gene in brain cortex did not demonstrate analogous differences[25]. Under physiological conditions, the activity of 5HT transporters in brain cortex also seems to be similar between 5HT-sublines (unpublished results). However, constitutively altered brain

functioning, as demonstrated by differences in brain 5HT turnover (unpublished results), in extrasynaptic 5HT concentration[21], and in response to pharmacological challenges[21] between the sublines, strongly suggest differentially regulated brain 5HT transmission, possibly involving 5HT receptors.

Thus, this study was aimed to compare the expression of 5HT-1A and 1B receptors, as important regulators of 5HT nerotransmission[12,32], in brains of Wistar-Zagreb 5HT rats. Expression of both receptor genes was studied in regions where they are abundantly expressed – 5HT--1A receptor in hippocampus, and 5HT-1B receptor in striatum. In order to obtain more reliable results, expression of target genes was related to three different reference genes in parallel. We have shown that messages for both 5HT-receptor genes under study are quite similar between the sublines, notwithstanding reference gene used to normalise expression signals.

Expression of 5HT-1A and 5HT-1B receptor genes has been explored in mice with genetically inactivated 5HTt gene, demonstrating region-specific changes in the expression of 5HT-1A and 5HT-1B receptor mRNAs[33,34]. Fabre et al. (2001) have shown marked reduction in 5HT-1A mRNA levels in raphe region, in contrast to higher level of this transcript measured in hippocampus[33], with the expression of 5HT-1B receptor gene not differing between knockout and wild type mice[33]. Similarly, Li et al. (2000) have found significant decrease of 5HT-1A mRNA level only in dorsal raphe area, while mRNA levels in hypothalamus remained unchanged when compared to wild type animals[34]. Electrophysiological studies on 5HTt knockout mice reported regional differences (raphe *vs* hippocampus) in pharmacologically induced 5HT-1A receptor desensitization[35]. In addition, chronic treatment of rats with selective 5HT reuptake inhibitor, fluoxetine, resulted in region-specific adaptation of 5HT-1A and 5HT-1B receptor, at both mRNA[36] and protein function level[36,37].

All these findings suggest that the adaptational changes in 5HT-1A and -1B receptors could have been expected in our animal model. The observed absence of differences in their expression between sublines might be due to several reasons.

First, changes could have occurred only at the receptor protein level, without altering mRNA expression. Similarly, Fabre et al. (2000) found significant decrease in 5HT-1B receptor labelling in 5HTt knockout mice, while levels of 5HT-1B mRNA revealed no differences. In this case, post-transcriptional steps accounted for the mentioned down-regulation of 5HT-1B receptors[33].

Secondly, a kind of robust alteration in brain neurochemistry, which could be induced by gene knockout, pharmacological challenge or stressful event, might be needed to provoke measurable changes in the adaptation of receptors. Thus, in contrast to our model, 5HTt knockout mice showed marked reductions in 5HT concentrations in various brain regions, while levels in heterozygous mice remained unchanged[38]. Literature data repeatedly show influence of stress on the expression of 5HT-1A receptors[39,40,41], at both mRNA and protein level, as well as on the expression of 5HT-1B receptors[42,43].

Finally, the present study was aimed at the postsynaptic 5HT receptors, while the literature data reported notable changes in the presynaptic receptors of the raphe region.

Our future studies will be aimed to the possible changes of the expression of 5HT-1A and 5HT-1B autoreceptors in raphe nuclei, as well as changes of the protein abundance/function (i.e. Western blot and/or receptor binding studies), both in physiological conditions and after a challenge. Once the nature of the changes in the central 5HT homeostasis of Wistar-Zagreb 5HT rats is defined, the sublines might represent a useful model of animals with constitutionally up- and down-regulated 5HT neurotransmission, and could find applications in different fields of 5HT-related research.

Acknowledgements

The results from this study are published within the scope of the projects »Serotonergic neurotransmission: genes, proteins and behavior«, no. 098-1081870-2395, and »Serotonergic mechanisms in alcoholism«, no. 098-1081870-2397, both supported by Croatian Ministry of Science, Education and Sport.

REFERENCES

1. LANFUMEY L, HAMON M, Curr Drug Targets, 3 (2004) 1. — 2. HENSLER JG, Life Sci, 72 (2003) 1665. — 3. PUCADYIL TJ, KALIPATNAPU S, CHATTOPADHYAY A, Cell Mol Neurobiol, 25 (2005) 553. — 4. GROSS C, ZHUANG X, STARK K, RAMBOZ S, OOSTING R, KIRBY L, SANTARELLI L, BECK S, HEN R, Nature, 416 (2002) 396. — 5. NEUMAISTER A, YOUNG T, STASTNY J, Psychopharmacol, 174 (2004) 512. — 6. CELADA P, PUIG MV, AMARGOS-BOSCH M, ADELL A, ARTIGAS F, J Psychiat Neurosci, 29 (2004) 252. — 7. BARNES NM, SHARP T, Neuropharmacol, 38 (1999) 1083. — 8. RAYMOND JR, MUKHIN YV, GELASCO A, TURNER J, COLLINSWORTH G, GETTYS TW, GREWAL JS, GARNOVSKAYA MN, Pharmacol Ther, 92 (2001) 179. — 9. SHARP T, HJORTH S, J Neurosci Meth, 34 (1990) 83. — 10. EL MANSARI M, BLIER P, Rev Psychiat Neurosci, 30 (2005) 268. — 11. TOTH M, Eur J Pharmacol, 463 (2003) 177. — 12. OLIVIER B, VAN OORSCHOT R, Eur J Pharmacol, 526 (2005) 207. — 13. SARI Y, Neurosci Biobehav Rev, 28 (2004) 565. — 14. ASE AR, READER TA, HEN R, RIAD M, DESCARRIES L, J Neurochem, 78 (2001) 619. — 15. ROCHA BA, SCEARCE-LEVIE K, LUCAS JJ, HIROI N, CASTANON N, CRABBE JC, NESTLER EJ, HEN R, Nature, 393 (1998) 175. — 16. CLARK MS, NEUMAIER JF, Psychopharmacol Bull, 35 (2001) 170. — 17. JERNEJ B, CICIN-SAIN L, Psychiat Res, 32 (1990) 67. — 18. JERNEJ B, HRANILOVIC D, CICIN-SAIN L, Neurochem Int, 33 (1999) 519. — 19. HRANILOVIC D, HERAK-KRAMBERGER CM, CICIN-SAIN L, SABOLIC I, JERNEJ B, Life Sci, 69 (2001) 59. — 20. HRANILOVIC D, CICIN-SAIN L, BORDUKALO T, JERNEJ B, Am J Med Gen, 114 (2002) 883. — 21. ROMERO L, JERNEJ B, BEL N, CICIN-SAIN L, CORTES R, ARTIGAS F, Synapse, 28 (1998) 313. — 22. HRANILOVIC D, CICIN-SAIN L, BORDUKALO-NIKSIC T, JERNEJ B, Behav Brain Res, 165 (2005) 271. — 23. QUEVEDO G, MOSCOSO O, PRADO-MORENO A, CICIN-SAIN L, JERNEJ B, DELGADO-GARCIA JM, Behavioral and molecular studies in rats over-expressing

and under-expressing serotonin transporter. In: COST B10 Meeting (2002). — 24. CICIN-SAIN L, BORDUKALO-NIKSIC T, JERNEJ B, Wistar-Zagreb 5HT rat: a new animal model of alcoholism. In: J Alcohol Relat Addict, suppl. 40 (2004) 92. — 25. BORDUKALO-NIKSIC T, CICIN-SAIN L, JERNEJ B, Neurosci Lett, 369 (2004) 44. — 26. JERNEJ B, CICIN-SAIN L, ISKRIC S, Life Sci, 43 (1988) 1663. — 27. JERNEJ B, FROEBE A, HRANILOVIC D, CICIN-SAIN L, Neurosci Res Commun, 24 (1999), 163. — 28. CHOMCZYNSKI S, SACCHI N, Anal Biochem, 162 (1987) 156. — 29. KULIKOV AV, NAUMENKO VS, VORONOVA IP, TIKHONOVA MA, POPOVA NK, J Neurosci Meth, 141 (2005) 97. — 30. LIU XY, WU SX, WANG YY, WANG W, ZHOU L, LI YQ, Neurosci Lett, 375 (2005) 42. — 31. CICIN-SAIN L, FROEBE A, BORDUKALO-NIKSIC T, JERNEJ B, Life Sci, 77 (2005) 452. — 32. ALBERT PR, LEMONDE S, Neuroscientist, 10 (2004) 575. — 33. FABRE V, BEAUFOUR C, EVRARD A, RIOUX A, HANOUN N, LESCH KP, MURPHY DL, LANFUMEY L, HAMON M, MARTRES MP, Eur J Neurosci, 12 (2000) 2299. — 34. LI Q, WICHEMS C, HEILS A, LESCH KP, MURPHY DL, J Neurosci, 20 (2000) 7888. — 35. MANNOURY LA COUR C, BONI C, HANOUN N, LESCH KP, HAMON M, LANFUMEY L, J Neurosci, 21 (2001) 2178. — 36. LE POUL E, BONI C., HANOUN N, LAPORTE AM, LAARIS N, CHAUVEAU J, HAMON M, LANFUMEY L, Neuropharmacol, 39 (2000) 110. — 37. NEWMAN ME, SHALOM G, RAN A, GUR E, VAN DE KAR LD, Eur J Pharmacol, 486 (2004) 25. — 38. BENGEL D, MURPHY DL, ANDREWS AM, WICHEMS CH, FELTNER D, HEILS A, MÖSSNER R, WESTPHAL H, LESCH KP, Mol Pharmacol, 53 (1998) 649. — 39. LOPEZ JF, CHALMERS DT, LITTLE KY, WATSON SJ, Biol Psychiat, 43 (1998) 547. — 40. LOPEZ JF, LIBERZON I, VASQUEZ DM, YOUNG EA, WATSON SJ, Biol Psychiat, 45 (1999) 934. — 41. BRIONES-ARANDA A, ROCHA L, PICAZO O, Prog Neur Psychopharmacol Biol Psychiat, 29 (2005) 275. — 42. NEUMAIER JF, EDWARDS E., PLOTSKY PM, Biol Psychiat., 51 (2002) 902. — 43. KAIYALA KJ, VINCOW ES, SEXTON TJ, NEUMAIER JF, Pharm Biochem Behav, 75 (2003) 769

L. Čičin-Šain,

Laboratory of Neurochemistry and Molecular Neurobiology, Molecular Biology Department, Rudjer Boskovic Institute, Bijenicka 54, HR-10000 Zagreb, Croatia
e-mail: cicinsai@irb.hr

EKSPRESIJA GENA ZA 5HT-1A I 5-HT-1B RECEPTORE U MOZGU WISTAR-ZAGREB 5HT ŠTAKORA

S A Ž E T A K

Usmjerenom selekcijom prema ekstremnim vrijednostima brzine trombocitnog serotoninskog prijenosnika (5HTt) razvijene su dvije sublinije štakora s visokom odnosno niskom vrijednošću toga parametra, nazvane »Wistar-Zagreb 5HT štakori«. Prethodna istraživanja pokazala su značajne razlike između sublinija u ekspresiji trombocitnog 5HTt, na razini i mRNA i proteina. U mozgu sublinija nisu pokazane analogne razlike u ekspresiji gena za 5HTt, iako farmakološke studije pokazuju jasne funkcionalne promjene 5HT prijenosnika u ventralnom hipokampusu koje upućuju na različitu homeostazu centralnog serotonina kod 5HT-sublinija. U ovom radu je istražena ekspresija gena za dva podtipa 5HT receptora: 5HT-1A receptor u hipokampusu i 5HT-1B receptor u strijatumu 5HT-sublinija. Metodom semi-kvantitativnog RT-PCR, provedenog uz tri različita referentna gena, nisu pokazane razlike u ekspresiji navedenih receptorskih gena. Rezultati upućuju da konstitucijske promjene 5HT homeostaze, nastale kao posljedica selektivnog odabira životinja prema aktivnosti 5HT prijenosnika, nisu dovele do promjena u ekspresiji postsinaptičkih 5HT-1A, odnosno 5HT-1B receptora kod Wistar-Zagreb 5HT štakora u fiziološkim uvjetima.