PERIODICUM BIOLOGORUM
VOL. 113, No 1, 81–86, 2011

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Original scientific paper

Physiological consequences of perinatal treatment of rats with 5-hydroxytryptophan

SOFIA BLAŽEVIĆ
PETRA DOLENEC
DUBRAVKA HRANILOVIĆ

Department of Animal Physiology
Faculty of Science,
University of Zagreb
Rooseveltov trg 6
HR-10 000 Zagreb, Croatia

Correspondence:
Dubravka Hranilović
Department of Animal Physiology
Faculty of Science
University of Zagreb
Rooseveltov trg 6
HR_10 000 Zagreb, Croatia
E-mail:dubravka@biol.pmf.hr

Key words: Serotonin (5-hydroxytryptamine, 5HT), 5-hydroxytryptophan (5HTP), Perinatal Rats, Physiology

Abbreviations:

5HT	5-hydroxytryptamine (Serotonin)
5HTP	5-hydroxytryptophan
Trp	Tryptophan
G	Gestation day
PND	Postnatal day

Received September 27, 2010.

Abstract

Background and Purpose: *Serotonin (5-hydroxytryptamine, 5HT) is present in brain and peripheral tissues and mediates various physiological functions. It also regulates perinatal development of serotonergic neurons and target tissues. It is assumed that dysregulation of the peripheral 5HT--homeostasis, which causes elevated blood 5HT concentrations, could inhibit development of serotonergic neurons and lead to anatomical/functional alterations of the brain. In this study we have investigated the physiological consequences of perinatal treatment with the immediate 5HT precursor, 5-hydroxytryptophan (5HTP) in young rats.*

Materials and Methods: *Rats were treated with 25 mg/kg 5HTP from gestational day 13 until postnatal day 21. The number of born and survived pups in each litter, body mass increase over time, level of anxiety produced by separation of pups from their mother, and blood 5HT concentrations were determined in the experimental group of rats and compared with values obtained in the saline-treated control group.*

Results: *Although a similar number of pups were born to each litter in both groups, 5HTP-treated pups, in comparison with saline-treated pups, had significantly lower body mass at PND1, significantly lower survival rate, significantly higher blood 5HT concentrations, and returned to their dam significantly faster in the separation anxiety test. They gained weight at slower rate than the control rats and maintained significantly lower body mass.*

Conclusion: *Temporary increase in peripheral 5HT concentrations during the critical phase of brain development has caused physiological disturbances in pups. Possible permanent changes in the central 5HT compartment are also indicated and will be explored in further studies.*

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5HT) is a biologically active amine present both in the brain and the peripheral tissues where it mediates various physiological functions *(1)*. Before it assumes the function of a neurotransmitter in the mature brain, it regulates the perinatal development of serotonergic neurons and target tissues *(2)*. Alterations in the system that regulates 5HT metabolism and function might therefore represent a biological basis of several behavioral disorders *(3)*.

A disorder in which 5HT homeostasis is disturbed both centrally and peripherally is autism, a neurodevelopmental syndrome with onset in early childhood, characterized by impairment in social interaction and communication, and by the presence of restricted and repetitive behaviors and interests *(4)*. Elevated blood 5HT levels (hyperserotone-

mia) have been consistently found in about one third of autistic patients *(5),* while at the same time brain 5HT activity was found to be decreased *(6)*.

Although 5HT is synthesized in the central and peripheral compartments via different tryptophan hydroxylase enzymes *(7)* and its two pools are separated by the blood-brain barrier, proteins that control 5HT function in both compartments are encoded by the same genes, have identical primary structures and follow the same kinetics *(8–10)*. It is therefore possible that alterations in the expression of one or more of the serotonergic elements could lead to the dysregulation of 5HT transmission in the brain, affecting so its early development and resulting in autistic behavioral symptoms, while it is at the same time reflected in the periphery as hyperserotonemia *(11)*.

Alternatively, dysregulation of the peripheral 5HT--homeostasis could lead to high concentrations of 5HT in blood. During fetal development, before the formation of the blood-brain barrier, these high 5HT levels could inhibit development of serotonergic neurons and lead to the anatomical and functional alterations of the brain, characteristic for autism *(12)*. Inhibitory function of 5HT on development of serotonergic neurons has so far been investigated on animal models using pharmacological treatment with 5HT receptor agonist 5-methoxytryptamine *(13, 14),* 5HT precursor tryptophan *(15),* monoamine oxydase inhibitors *(16),* and 5HT uptake inhibitors *(17)*.

We have recently started studies of the effects of hyperserotonemia on the developing rat brain by administering immediate 5HT precursor, 5-hydroxytryptophan (5HTP) perinatally from gestational day 13 until postnatal day 21, the period of most intensive development of serotonergic neurons. With the hypothesis that the mentioned treatment will cause hyperserotonemia and lead to measurable physiological consequences in young rats, we have determined the number of born and survived pups in each litter, body mass increase over time, anxiety-like behavior of pups and blood 5HT concentrations in rats treated with 25 mg/kg 5HTP and compared the measured parameters with those of the saline treated control rats.

MATERIALS AND METHODS

Housing and breeding of animals

Out of five nulliparous Wistar females from the animal facility of the Croatian Institute for Brain Research (University of Zagreb, Zagreb, Croatia), weighing 260–291 g, two were assigned to a »saline group«, and three to a »5HTP group«. Females were mated with males of the same strain and age in 2:1 and 3:1 ratio, respectively. Vaginal smears were taken daily at 10 a.m. to check for the presence of sperm. Weight was monitored daily and progressive increase during the following week was considered as a confirmation of pregnancy. The day sperm was found in the smear was considered as day 0 of gestation (G0). After gravidity was confirmed in all females, the male was removed from the cage. Females remained together until two days before parturition when they were separated and remained singly housed until weaning of the pups (at postnatal day 21, PND 21). After weaning, animals were kept 3 per cage. Females were closely observed during parturition to determine the number of pups born to each litter. Pups were weight daily during treatment and three times weekly after treatment. Animals were housed in polycarbonate cages under 12-h light:12-h dark conditions at a temperature of 22 ± 2 °C, with free access to rat chow and tap water. The study was approved by the Ethic committee of the Faculty of Science, University of Zagreb, and was conducted in accordance with the Croatian Animal Protection Law (»Narodne novine«, 135/2006).

Pharmacological treatment

The experimental group of pups was treated with 5HTP (Sigma-Aldrich), from GD 13 until birth by injecting 25 mg/kg of 5HTP subcutaneously to pregnant females, and from PND1 until PND 21 by receiving subcutaneous injections in the nape at a dose 25 mg/kg. 5HTP was dissolved in acidified saline. Before treatment, the solution was neutralized with NaOH and warmed to the body temperature. The control group was treated with saline in the same manner. All injections were performed at 2 pm. A 50 μL syringe (Hamilton) and disposable 30G needles (BD, Drogheda, Ireland) were used to treat the pups.

Behavioral test – Return to dam

The return to dam test was adapted from McNamara *et al. (14)*. The test was performed on PND 17 in a cage with a dark non translucent wall inserted in the middle. The wall contained a 2.5 × 2.5 cm opening at the bottom with a tunnel-like extension on the mother's side, so the pups could pass but she could not reach out for them. A maximum of 5 pups per litter were placed on one side of the wall and the dam on the other. The pups were allowed ten minutes to return to their dam and the time when their hind legs crossed through the opening in the wall onto the mother's side was scored.

Blood 5HT concentration

Blood 5HT concentrations were measured at the end of the treatment (PND 22) in five randomly chosen pups from each treatment group. Under light ether narcosis, 800 μL of blood was withdrawn from the jugular vein into syringes preloaded with 200 μL of 3.13% trisodium citrate anticoagulant. Animals were sacrificed after blood sampling. Blood samples were transferred to microtubes after a thorough mixing and centrifuged at 200 × g for 10 min to generate platelet rich plasma. 5HT concentration in both, platelets and platelet-free plasma was determined using a commercial enzyme immunoassay kit (Serotonin ELISA kit, DRG Instruments GmbH, Germany), according to the kit instructions. A calibration curve was drawn based on the absorbances measured at 450 nm on the microplate reader (Bio Rad 550, Germany) and known concentrations of the standard solutions. Concentration values of samples were obtained by

interpolating them to the calibration curve, using the 4-parameters non-linear regression curve fitting. Results were counted as a sum of concentration in platelets and concentration in platelet-free plasma, and were expressed in ng 5HT per mL of blood.

Statistical analysis

Data was processed using GraphPad InStat 3.01 software. Normality of distributions of the measured parameters was tested by Kolmogorov/Smirnov method, while the equality of SDs was tested by Bartlett's test. Mean values of normally distributed parameters were compared using unpaired t-test, and of those that were not normally distributed using non-parametric Mann-Whitney test. Statistical significance of difference in survival rate was compared using two-sided Fisher's exact test. The level of significance was set to 0.05. Values were expressed as means ± standard deviations (M ± SD).

RESULTS

Several physiological parameters were determined in rats perinatally treated with the serotonin precursor 5HTP and compared to those of the saline treated rats (Table 1).

Two dams from the control group gave birth to 10 and 9 pups, respectively, one of which died during the first 24 hrs. Three dams from the 5HTP treated group gave birth to a total of 24 pups (8 per dam) out of which only ten survived after the first 24 hrs. The difference in survival rate was very significant (p = 0.0003), with a relative risk of dying for the 5HTP treated pups being 2.6 (95% CI 1.6–4.4).

Although the number of pups born per dam (9.5 in saline treated and 8 in 5HTP treated group), as well as the maternal weight gain during pregnancy (121,9% in the saline treated and 109,7% in the 5HTP treated group), was very similar between the groups, body mass of the surviving pups on PND 1 was significantly lower in the 5HTP treated (6.0 ± 0.7 g) than in the saline treated (7.3 ± 0.8 g) group (t = 4.222, df = 22, p = 0.0004) (Figure 1).

TABLE 1

Physiological parameters determined in saline- and 5HTP-treated rats.

	Saline treated group	5HTP treated group
Number of pups born	19	24
Offspring per dam	9.5	8
Number of died pups	1	14
Litter size per dam	9	3.33 ± 0.58
Survival rate (%)	95	42 ###
Birth weight (g)	7.3 ± 0.8	6.0 ± 0.8 ***
Adult weight at PND 44 (g)	162.0 ± 17.5	140.6 ± 22.4 **

** p<0.01, *** p<0.001, unpaired t-test; ### p<0.001, Fisher's exact test.

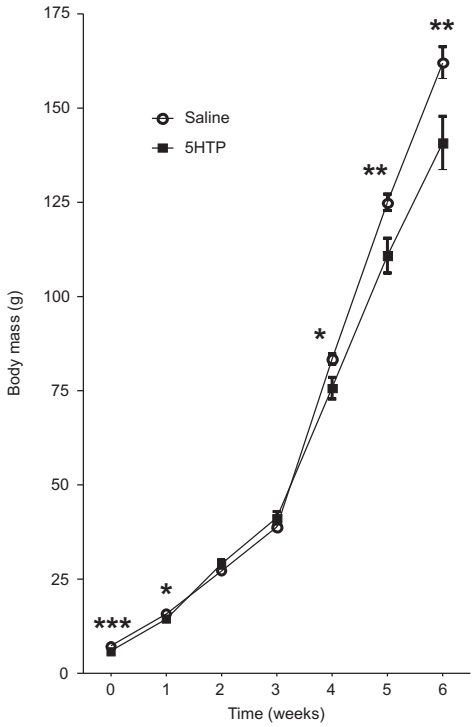

Figure 1. *Increase in body mass in rats perinatally treated with saline N=17 (circles), or 5HTP N= 10 (squares). Values are expressed as M ± SD. Differences in mean values of the body mass between the groups were compared at different time points using unpaired t-test; **p<0.01, *** p<0.001, p<0.05.*

Weight gain during breast-feeding period was constant in both groups and the average body mass of 5HTP treated pups reached that of saline treated pups after the second week of age (PND 15, 29.09 ± 1.080 g and 27.34 ± 0.3086 g, respectively), probably due to much smaller litter sizes. However, after weaning, the body mass of the 5HTP treated rats increased at a slower rate than the body mass of the saline treated rats, resulting in a significantly lower weight at adult age (PND 44, 140.6 ± 22.4 g and 162.0 ± 17.5 g, respectively; U = 32; p = 0.0067).

On PND 17, possible differences in behavior between the saline and 5HTP treated pups were determined by the return-to-dam test (Figure 2). While the saline trea-

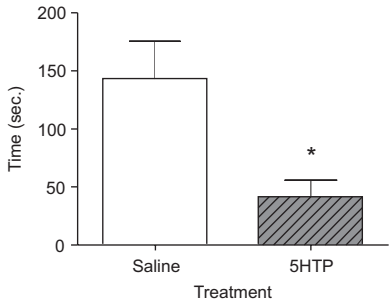

Figure 2. *Return to dam test. Bars show time needed for saline (N=18) and 5HTP (N=10) treated pups to return to their dam after separation. Values are expressed as M ± SD; * p<0.05, Mann Whitney test.*

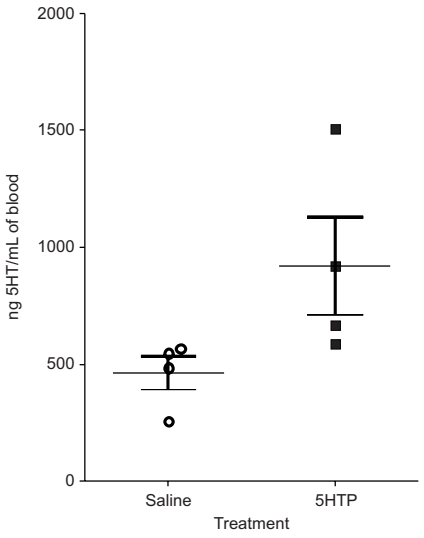

Figure 3. *Blood 5HT concentrations in rats perinatlly treated with saline or 5HTP at the end of treatment (PND 22). N=4 per group. Values are expressed as M ± SD; * p<0.05, Mann Whitney test.*

ted pups needed 143 ± 32 s on average to return to their mothers, the 5HTP treated pups performed the given task significantly faster needing on average only 41 ± 14 s (U = 37; p = 0.0118).

Finally, in order to determine whether the perinatal 5HTP treatment did indeed cause hyperserotonemia in the experimental group, blood 5HT concentrations were measured in five randomly chosen pups from both groups at the end of treatment. One sample from each group was lost during processing, leaving us with four samples per group (Figure 3). Although the number of samples was low, it was evident that pups from the 5HTP treated group have elevated blood 5HT concentrations in comparison to those of the saline treated group, mean values being 463 ± 142 ng/mL and 920 ± 416 ng/mL, respectively (U=16, p=0.0286).

DISCUSSION

By the use of the described method, we have caused hyperserotonemia in the experimental group of rats, with the mean blood 5HT concentration amounting to about 200% of the mean 5HT concentration in the saline treated group. In the body, 5HTP is the intermediate in the synthesis of 5HT from its precursor tryptophan (Trp) through a rate-limiting step mediated by the enzyme tryptophan hydroxylase. 5HTP is then reduced by aromatic amino acid decarboxylase to serotonin. Although Trp has been used by some groups to increase 5HT levels perinatally *(18–20),* we consider the use of 5HTP to have several advantages. First, Trp is an essential amino acid with many functions in the body (mainly a precursor in protein synthesis) while only 1–2% is consumed in the synthesis of 5HT *(21)*; on the other hand, 5HTP is only found in the 5HT synthesis pathway and is quantitatively converted to 5HT *(21–23)*. Secondly, administration of 5HTP allowed us to elude the rate-limiting step in the synthesis of 5HT and to mimic the effect of increased 5HT synthesis through a chosen 5HTP dose. Indeed, clinical studies have shown that 5HTP is effective in increasing blood 5HT levels *(24–27)* and is more effective than Trp in increasing brain 5HT levels *(28)*. The advantage of using 5HTP over 5HT itself is that it readily crosses the placental barrier *(21),* which is crucial for the prenatal part of the treatment. Hirai and Nakajima *(29)* have shown that a dose of 20 mg/kg 5HTP is minimal to cause a measurable increase in blood 5HT levels in Wistar rats, while maintaining a physiological proportion between 5HT and 5HIAA content. On the other hand, higher doses of 5HTP (100 mg/kg and above), although effective in rising 5HT blood levels, proved to be neurotoxic for rodents, causing the 5HT syndrome *(30–33)*. We have chosen a dose of 25 mg/kg 5HTP which is reported to be quite effective in raising blood 5HT concentrations in adult rats *(34–37)*. The injections were given subcutaneously in the nape both to gravid dams and pups. This way of drug administration, used to avoid the risk of damaging the fetuses during the prenatal treatment, and to reduce discomfort in the pups, enabled 100% of pup survival during treatment.

The physiological consequences of the perinatal treatment with 5HTP in young rats were evident. Although a similar number of pups were born to each litter in both, 5HTP treated and saline treated dams, pups from the 5HTP treated group had significantly lower body mass at PND1 and significantly lower survival rate. Pups which did not survive were either still born or died within 24 hours after birth. Research on the influence of 5HTP in pregnant rats, showed that an acute dose of 100 mg/kg of 5HTP caused reduced fetal weight and increased fetal reabsorption *(38),* presumably caused by the vasoconstricting effect of serotonin, especially on the umbilical and chorionic arteries. The negative effect of the reduced uteroplacental blood flow on fetal growth has been demonstrated in several studies *(39–41)*. The chronic treatment with 5HTP used in this experiment, although at a much lower dose, could have reduced placental blood flow and induced slower fetal growth and, consequently, lower survival rate. Another possible explanation might be that the increased 5HT concentrations caused by 5HTP treatment have impaired development of serotonergic brain regions, which was reflected in death of some pups and lower birth weight of others.

The influence of the 5HTP treatment on body mass was obvious after weaning. Free-feeding rats from the experimental group gained weight at slower rate than the control rats and retained significantly lower body mass after the wash-out period. This indicates that 5HTP treatment has induced changes in the central serotonergic compartment resulting in reduced food intake or increased metabolic rate. A number of studies reported the influence of centrally and peripherally increased 5HT concentrations on decreased food ingestion, and consequentially, lower body mass in both, rats and humans *(42–47)*.

The return to dam test was conducted in order to determine the level of anxiety produced by the separation of the pups from their mother. Although all pups from both groups returned to their dam within the experimental time, 5HTP treated pups did it significantly faster than the control pups. This might be the result of increased locomotor activity, increased anxiety, or the combination of both. Regarding the first, increased frequencies in basic locomotor patterns have been observed after the acute administration of 55 mg/kg of 5HTP in carbidopa pretreated rats *(48)*. Regarding the second, increased anxiety-like behavior was observed in a rat subline with high platelet 5HT level in comparison to the subline with low platelet 5HT level *(46)*. Also, the reduction in brain 5HT levels induced through tryptophan depletion has been reported to cause panic attacks and anxiety in patients with panic syndrome as well as in healthy subjects *(49, 50)*. Hence, it is possible that the results of our behavioral test represent the consequence of reduced activity and/or number of serotonergic neurons caused by peripheral 5HT increase after 5HTP treatment.

At this point it is hard to distinguish between the indirect 5HTP effects, acting through hyperserotonemia, and direct 5HTP effects acting through the increased 5HT synthesis in the brain, which represents the main limitation of the study. In any case, the brain was exposed to elevated 5HT levels during the development of serotonergic neurons.

In conclusion, we have shown that the perinatal treatment of rats with 25 mg/kg of the serotonin precursor 5HTP has caused physiological disturbances in pups. Differences in body mass between 5HTP and saline treated animals, which remained significant after the wash-out period, indicate that the changes in the central 5HT compartment might have been permanent. Whether the temporary increase in peripheral 5HT concentrations during the critical phase of brain development has left permanent changes in the central serotonergic compartment, and to what extent, will be explored in further studies.

REFERENCES

1. VANDER A J, LUCIANO D, SHERMAN J 2001 Human Physiology: The Mechanisms of Body Function, 8th Edition. McGraw-Hill, New York, p 207
2. WHITAKER-AZMITIA P M 2001 Serotonin and brain development: Role in human developmental diseases. *Brain Res Bull 56*: 479–485
3. LESCH K P, MOESSNER R 1998 Genetically driven variation in serotonine uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? *Biol Psychiat 44*: 179–192
4. OWLEY T, LEVENTHAL B L, COOK E H 2003 Childhood disorders: The autism spectrum disorders. *In*: Tasman A, Kay J, Lieberman JA *(ed)* Psychiatry, 2nd Edition. Wiley and Sons, West Sussex, England, p 757
5. COOK E H, LEVENTHAL B L 1996 The serotonin system in autism. *Curr Opin Pediatr 8*: 348–354
6. CHUGANI D C, MUZIK O, ROTHERMEL R, BEHEN M, CHAKRABORTY P, MANGNER T 1997 Altered serotonin synthesis in the dentatothalamocortical pathway in autistic boys. *Ann Neurol 42*: 666–669
7. WALTHER D J, PETER J U, BASHAMMAKH S, HORTNAGL H, VOITS M, FINK H, BADER M 2003 Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science* 299, 76
8. LESCH K P, AULAKH C S, WOLOZIN B L, TOLLIVER T J, HILL J L, MURPHY D L 1993 Regional brain exspression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressant. *Mol Brain Res 17*: 31–35
9. CHEN K, WU H F, SHIH J C 1993 The deduced aminoacid sequences of human platelet and frontal cortex monoamine oxidase B are identical. *J Neurochem 61*: 187–190
10. COOK E H, FLETCHER K E, WAINWRIGHT M, MARKS N, YAN S, LEVENTHAL B L 1994 Primary structure of the human platelet serotonin 5HT-2A receptor: Identity with frontal cortex serotonin 5HT-2A receptor. *J Neurochem 63*: 465–469
11. JANUSONIS S 2005 Serotonergic paradoxes of autism replicated in a simple mathematical model. *Med Hypotheses 64*: 742–750
12. WHITAKER-AZMITIA P M 2005 Behavioral and cellular consequences of increasing serotonergic activity during brain development: a role in autism? *Int J Dev Neurosci 23*: 75–83
13. SHEMER A V, AZMITIA E C, WHITAKER-AZMITIA P M 1991 Dose-related effects of prenatal 5-methoxytryptamine (5-MT) on development of serotonin terminal density and behavior. *Dev Brain Res 59(1)*: 59–63
14. MCNAMARA I M, BORELLA A W, BIALOWAS L A, WHITAKER-AZMITIA P M 2008 Further studies in the developmental hyperserotonemia model (DHS) of autism: Social, behavioral and peptide changes. *Brain Res 1189*: 203–214
15. HUETHER G, THOMKE F, ADLER L 1992 Administration of tryptophan-enriched diets to pregnanat rats retards the development of the serotonergic system in their offspring. *Dev Brain Res 68*: 175–181
16. WHITAKER-AZMITIA P M, ZHANG X, CLARKE C 1994 Effects of gestational exposure to monoamine oxidase inhibitors in rats: preliminary behavioral and neurochemical studies. *Neuropsychopharmacol 11(2)*: 125–132
17. CABERA-VERA T M, GARCIA F, PINTO W, BATTAGLIA G 1997 Effect of Prenatal Fluoxetine (Prozac) Exposure on Brain Serotonin Neurons in Prepubescent and Adult Male Rat Offspring. *J Pharmacol Exp Ther 280*: 138–145
18. HERNANDEZ-RODRIGUEZ J, CHAGOYA G 1986 Brain serotonin synthesis and Na+,K+-ATPase activity are increased postnatally after prenatal administration of L-tryptophan. *Brain Res 390(2)*: 221–226
19. MARTIN L, RODRIGUEZ DIAZ M, SANTANA-HERRERA C, MILENA A, SANTANA C 1997 Tryptophan ingestion by gestant mothers alters prolactin and luteinizing hormone release in the adult male offspring. *Brain Res 774*: 265–268
20. AREVALO R, ALFONSO D, CASTRO R, RODRIGUEZ M 1991 Fetal brain serotonin synthesis and catabolism is under control by mother intake of tryptophan. *Life Sci 49*: 53–66
21. BIRDSALL T C 1998 5-Hydroxytryptophan: a clinically-effective serotonin precursor. *Altern Med Rev 3*: 271–280
22. UDENFRIEND S, WEISSBACH H, BOGDANSKI DF 1957 Increase in tissue serotonin following administration of its precursor 5-hydroxytryptophan. *J Biol Chem 224(2)*: 803–810
23. MAGNUSSEN I, NIELSEN-KUDSK F 1980 Bioavailability and related pharmacokinetics in man of orally administered L-5-hydroxytryptophan in steady state. *Acta Pharmacol Tox 46(4)*: 257–262
24. TURNER E H, LOFTIS J M, BLACKWELL A D 2006 Serotonin a la carte: Supplementation with the serotonin precursor 5-hydroxytryptophan *Pharmacol Therapeut 109*: 325–338
25. VAN PRAAG H M 1982 Serotonin precursors in the treatment of depression. *Adv Biochem Psychoph 34*: 259–286
26. VAN PRAAG H M, KORF J, DOLS L C, SCHUT T 1972 A pilot study of the predictive value of the probenecid test in application of 5-hydroxytryptophan as antidepressant. *Psychopharmacologia 25(1)*: 14–21
27. ZMILACHER K, BATTEGAY R, GASTPAR M 1998 L-5-hydroxytryptophan alone and in combination with a peripheral decarboxylase inhibitor in the treatment of depression. *Neuropsychobiology 20(1)*: 28–35
28. THORRÉ K, SARRE S, TWAHIRWA E, MEEUSEN R, EBINGER G, HAEMERS A, MICHOTTE Y 1996 Effect of L-tryptophan, L-5-hydroxytryptophan and L-tryptophan prodrugs on the

extracellular levels of 5-HT and 5-HIAA in the hippocampus of the rat using microdialysis. *Eur J Pharm Sci 4*: 247–256

29. HIRAI M, NAKAJIMA T 1979 Biochemical studies on the mechanism of difference in the renal toxicity of 5-hidroxy-L-tryptophan between Sprague Dawley and Wistar rats. *J Biochem 86*: 907–913
30. CASAL J A, CORZO M D, PEREZ L F, ALVAREZ J A, ALDEGUNDE M, TUTOR J C 2000 Pharmacological modification of the serotonergic transmitter system and beta-N-acetylhexosaminidase activity in rats. *Life Sci 67*: 2369–2374
31. MOKLER D J, SULLIVAN S A, WINTERSON B J 1992 Behaviors induced by 5-hydroxytryptopahn in neonatal, preweaning, postweaning, and adult Sprague-Dawley rats. *Pharmacol Biocem Beh 42(3)*: 413–419
32. NISIJIMA K, YOSHINO T, YUI K, KATOH S 2001 Potent serotonin (5-HT)(2A) receptor antagonists completely prevent the development of hyperthermia in an animal model of the 5-HT syndrome. *Brain Res 890*: 23–31
33. NISIJIMA K, YOSHINO T, ISHIGURO T 2000 Risperidone counteracts lethality in an animal model of the serotonin syndrome. *Psychopharmacology 150(1)*: 9–14
34. ENGLEMAN E A, MURPHY J M, ZHOU F C, APRISON M H, HINGTGEN J N 1995 Operant response suppression induced with systemic administration of 5-hydroxytryptophan is centrally mediated. *Pharmacol Biocem Beh 52(3)*: 525–529
35. JOYCE D, HURWITZ H M B 1964 Avoidance behaviour in the rat after 5-hydroxytryptophan (5-HTP) administration. *Psychopharmacologia 5*: 424–430
36. PARK W K, HINGTGEN J N, APRISON M H 1991 Differential effect of 5-hydroxytryptophan on approach and avoidance behavior in rats. *Pharmacol Biocem Beh 38(1)*:191–194
37. PENN P E, MCBRIDE W J, HINGTGEN J N, APRISOn M H 1977 Differential uptake, metabolism and behavioral effects of the D and L isomers of 5-hydroxytryptophan *Pharmacol Biocem Beh 7(6)*: 515–518
38. SALAS S P, GIACAMAN A, ROMERO W, DOWNEY P, ARANDA E, MEZZANO D, VÍO C P 2007 Pregnant rats treated with a serotonin precursor have reduced fetal weight and lower plasma volume and kallikrein levels. *Hypertension 50*: 773–779
39. DUVEKOT J J, CHERIEX E C, PIETERS F A A, MENHEERE P P C A, SCHOUTEN H J A, PEETERS L L H 1995 Maternal volume homeostasis in early pregnancy in relation to fetal growth restriction. *Obstet Gynecol 85*: 361–367
40. SALAS S P, ROSSO P 1998 Plasma volume, renal function, and hormonal levels in pregnant women with idiopathic fetal growth restriction or preeclampsia. *Hypertens Pregnancy 17*: 69–79
41. SALAS S P, MARSHALL G, GUTIERREZ B L, ROSSO P 2006 Time course of maternal plasma volume and hormonal changes in women with preeclampsia or fetal growth restriction. *Hypertension 47*: 203–208
42. BROQUA P, BAUDRIE V, CHAOULOFF F 1992 Differential effects of the novel antidepressant tianeptine on L-5-hydroxytryptophan (5-HTP)- elicited corticosterone release and body weight loss. *Eur Neuropsychopharm 2(2)*: 115–120
43. CURZON G 1990 Serotonin and apetite. *Ann NY Acad Sci 600*: 521–530
44. FLETCHER P J 1988 Increased food intake in satiated rats induced by the 5HT antagonists methysergide, metergoline and ritanserin. *Psychopharmacology 96*: 237–242
45. HALFORD J C, HARROLD J A, LAWTON C L, BLUNDELL J E 2005 Serotonin (5-HT) drugs: effects on appetite expression and use for the treatment of obesity. *Curr Drug Targets 6*: 201–213
46. HRANILOVIĆ D, ČIČIN-ŠAIN L, BORDUKALO-NIKŠIĆ T, JERNEJ B 2005 Rats with constitutionally upregulated/downregulated platelet 5HT transporter: Differences in anxiety-related behavior. *Behav Brain Res 165*: 271–277
47. POLLOCK J D, ROWLAND N 1981 Periphetally administered serotonin decreases food intake in rats. *Pharmacol Biocem Beh 15*: 179
48. CLARKE K A, PARKER A J, STIRK G C 1984 Locomotion in the rat after 5-hydroxy-L-tryptophan. *Eur J Pharm Sci 98(2)*: 255–260
49. MILLER H E, DEAKIN J F, ANDERSON I M 2000 Effect of acute tryptophan depletion on CO2-induced anxiety in patients with panic disorder and normal volunteers. *Brit J Psychiat 176*: 182–188
50. SCHRUERS K, KLAASSEN T, POLS H, OVERBEEK T, DEUTZ N E, GRIEZ E 2000 Effects of tryptophan depletion on carbon dioxide provoked panic in panic disorder patients. *Psychiat Res 93*: 179–187