ejpmr, 2019,6(11), 527-532

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

A COMPARATIVE STUDY OF TRYPTOPHAN HYDROXYLASE'S CIRCADIAN RHYTHM IN THE FUNCTIONAL PARTS OF DORSAL RAPHE NUCLEI IN THE MESENCEPHALON

Zeina Malek[1] and Louay Labban*[2]

[1]Faculty of Pharmacy, Syrian Private University, Syria.
[2]Faculty of Pharmacy, Al Jazeera Private University, Syria.

***Corresponding Author: Dr. Louay Labban**
Faculty of Pharmacy, Al Jazeera Private University, Syria.

Article Received on 21/09/2019 **Article Revised on 11/10/2019** **Article Accepted on 01/11/2019**

ABSTRACT

Dorsal Raphe (DR), the most developed part of the Raphe complex, is divided into three cellular well defined subnuclei: the ventromedian (VM), the dorsomedian (DM), and the lateral (L). Also, the raphe complex is known to exhibit circadian variations within numerous processes concerning synthesis and release of Serotonin, its main neurotransmitter. On the other hand, it has been established that the biological clock of the Suprachiasmatic Nuclei (SCN), controls the circadian behavior of the Serotonin neurons. In this study, the rate limiting enzyme in serotonin synthesis, Tryptophan Hydroxylase, was measured within all the DR subdivisions among which only the lateral parts showed circadian variations. Our findings suggest a differential regulation in Serotonin synthesis at the cellular levels of DR, taking in consideration that the lateral parts were thought to be the most involved subdivision in the circadian system, both upon anatomical and functional levels.

KEYWORDS: Tryptophan hydroxylase, Biological Clock, Circadian rhythm, Dorsal raphe.

1- INTRODUCTION

Synthesized from the essential amino acid Tryptophan, the neurotransmitter Serotonin is a monoamine that interferes with many important brain functions such as sleep/wake cycle, alert system, dietary behavior and learning.[1-2] Also, reduced serotonin release in the synapse is directly related to the onset of depression symptoms.[2-3] In the brain, nerve nuclei that synthesize the neurotransmitter serotonin are concentrated in the midbrain region, in the Raphe Nuclei Complex (RN).[4] Hypothalamus receives a high-density neural connection from the Raphe nuclei, one of the most prominent and most closely related hypothalamic targets, are the suprachiasmatic Nuclei (SCN)[4-8], the locus of the circadian clock.[9-11] The circadian characteristics of serotonergic neurons have been previously well defined. The circadian behavior of Raphe nuclei has been studied for both the synthesis and release of serotonin.[12-15] Tryptophan Hydroxylase (TPH, the rate limiting enzyme in serotonin synthesis), and the mRNA levels of this enzyme both exhibit day-to-day changes in critical parts of the circadian system. These changes persist when rats are housed in constant darkness for a specified period of time, indicating their internal origin and their control by the biological clock. The protein synthesis of this enzyme has been site-calibrated on specific parts of the Raphe nuclei thought to be directly related to the components of the circadian system, the ventral nucleus of the Raphe complex (Median Raphe, MR) known for its anatomically close contact with the biological clock.[13-14] Studies have indicated that the circadian variations of serotonin-containing cells are under the influence of the biological clock, mediated by glucocorticoids.[16]

In this study, we checked another component of the Raphe complex, the dorsal nuclei (Dorsal Raphe, DR), which are anatomically complex and known nuclei, such as in MR, interfering with the daily system through anatomical connections with several brain circadian structures.[4-8] The protein synthesis of tryptophan hydroxylase was calibrated at the site throughout the DR sub-nuclei to determine what the daily clock is practiced on these serotonergic neurons by using the technique of fixing the frozen brain tissue sections by thermal shock on nitrocellulose filters, allowing an accurate anatomical study of the whole Raphe DR.

2- MATERIALS AND METHODS

2-1: Animal models

In this study, Wistar male rats obtained from Faculty of Medicine, University of Strasbourg (France) were used. They were housed in typical conditions for the sequence of light and darkness at 12:12, where the lighting is adjusted automatically and the lighting period extends from 6 am to 18 pm (6:00 pm). 48 hours before cerebral

sampling, half of the rats were housed with constant darkness without any white light, to examine the effect of the biological clock on the concentrations of tryptophan hydroxylase, while the second half remained in light and dark cycle 12:12. Brain specimens were taken every 4 hours by decapitation, rapid dissection of the cranial area and extraction of the entire brain tissue Intact without any sabotage for a maximum of 30 seconds, the brain is then frozen by electrocution in the cooled isopentane solution in liquid nitrogen to -30 ° C for a minute and a half and then stored in the freezer at -80 ° C until the preparation of tissue sections.

2-2: Preparation of brain sections

Brain sections were prepared using a cooled precision cutter where the cutting chamber temperature is -20˚ C (Cryostat, Leica Instruments GmbH, Germany) and the thickness of the tissue sections is 20 µm. 10 histological levels were prepared along the entire anterior to posterior anatomical length of the dorsal Raphe nuclei within the anatomical extension of the midbrain, where the tissue section is placed directly on a Millipore HAHY filter and is associated with intermolecular forces. This leads to the simultaneous fixation of all proteins within this brain section in order to study them quantitatively. The results of the fixation of proteins on brain sections have been tested and validated in previous studies.

2-3: Immunoautoradiography for tryptophan hydroxylase enzyme

The sections installed on the nitrocellulose filters for 45 minutes are treated with Tris saline (TBS) at a concentration of 50 mmol and containing bovine serum albumin (Bovine Serum Albumin, BSA) at a concentration of 0.5% from Roche (TBS-BSA 0.5%) to saturate the nonspecific binding sites within the sample. The sections were then incubated with the presence of the antibody solution of tryptophan hydroxylase manufactured in the laboratories of Dr. Michel Maitre at the Faculty of Medicine of the University of Strasbourg (France)[19-21] by extending 1/250 into a 1% TBS-BSA solution. The next day, after three washings (3x10min) with 0.5% TBS-BSA solution, the sections were incubated for two hours in the presence of a radioactive iodine-labeled G protein (NEN, G [I-125]) by extending 1/500 into a 1% TBS-BSA solution that qualitatively interferes with antibodies. The sections were then washed with TBS gradient solutions (50 mmol with 0.5% BSA, 50 mmol, then 500 mmol), 10 minutes per wash and dried after passing with distilled water and then stacked for two days against Kodak Biomax films. The films were quantified and analyzed using the image reading software (Biocom, RAG2000) by measuring the optical density (OD) of the Raphe nuclei tagged along their anatomical anterior and posterior anatomical length and using the ANOVA (Analysis Of Variance) method for comparative statistical study.

3- RESULTS AND DISCUSSION

Fig. 1, shows the anatomical parts of the dorsal Raphe nuclei during the quantitative analysis of tryptophan hydroxylase, consisting of a central triangular section, the ventral central section (Ventromedian, VM), are shown on both sides of two lateral sections (Lateral, L), also called the lateral wings of DR, and finally the middle dorsal section (Dorsomedian, DM).

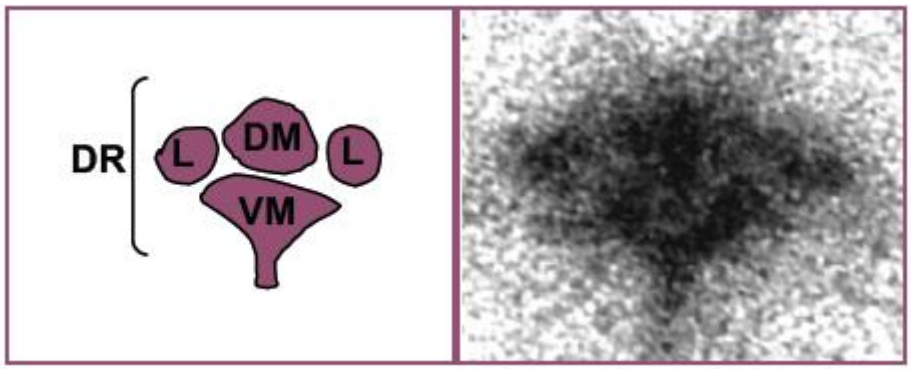

Figure 1: Detailed anatomical structure of the dorsal Raphea nuclei as shown in the autoimmunoradiography detection of tryptophan hydroxylase enzyme.

It appears to have the middle ventral part (VM), lateral parts (L) and the middle dorsal part (DM).

Measuring the optical density of tryptophan hydroxylase in the ventral middle section of rats that remained in typical lighting conditions for 12 hours versus 12 hours in the dark showed no systemic changes and semi-constant levels with day and night succession as shown in (Fig. 2).

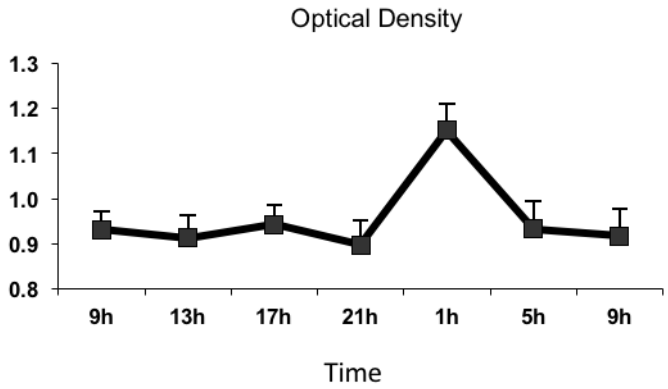

Figure 2: Daily changes in the concentrations of tryptophan hydroxylase enzyme represented by the optical density in the VM section of the dorsal Raphe nuclei of rats housed in light and dark succession 12:12 (*p> 0.05*). The light period extends from 6 to 18 hours.

Also, no change in the optical density values of tryptophan hydroxylase was observed in the middle ventral part of the dorsal Raphe nuclei of rats exposed to two-day complete darkness conditions (Fig. 3).

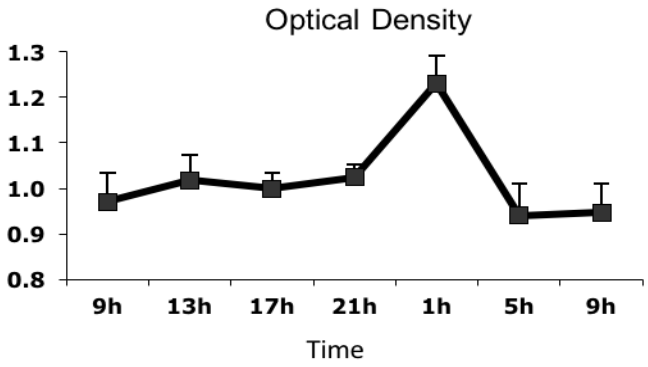

Figure 3: Daily changes in the concentrations of tryptophan hydroxylase enzyme represented by the optical density of the VM section of the dorsal Raphe nuclei in rats housed in continuous dark conditions for 48 h before sampling (*p> 0.05).*

On the other hand, within the lateral parts of DR (L, the lateral wings), the levels of this enzyme showed significant changes in both rats housed in typical lighting conditions (Fig. 4) and those that were left in complete darkness for two days (Fig. 5), indicating the internal origin of these changes subject to biological clock systems. These data are consistent with what was obtained in a previous study.[12]

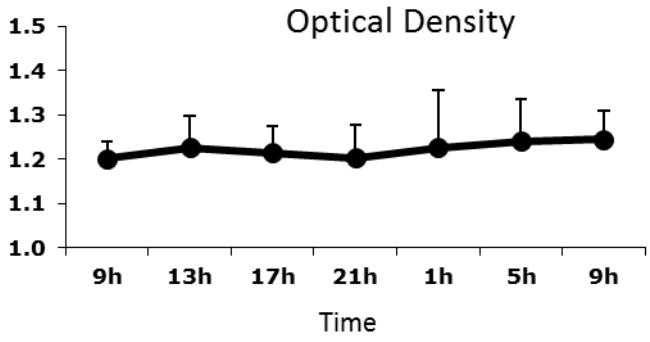

Figure 4: Daily changes in the concentrations of tryptophan hydroxylase enzyme represented by the optical density of the L section of the dorsal Raphe nuclei of rats housed in conditions of light and dark succession 12:12 (p <0.01, **) The light period extends from 6 to 18 o'clock.

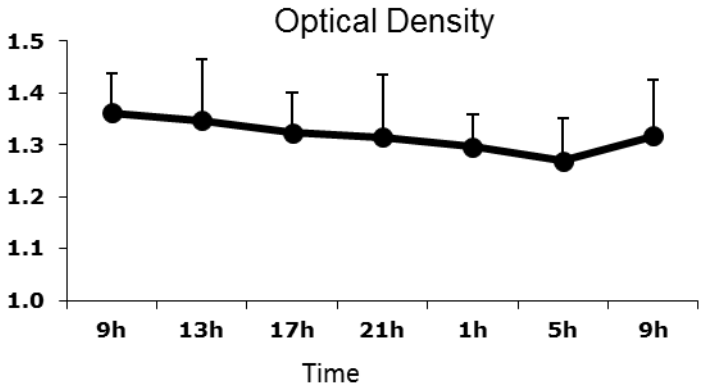

Figure 5: Daily changes in the concentrations of tryptophan hydroxylase enzyme represented by the optical density in the L section of the dorsal Raphe nuclei of rats exposed to continuous dark conditions for 48 h before sampling. The statistical differences were significant at (p <0.01. **).

No apparent changes to the circadian systems of tryptophan hydroxylase were observed in the middle dorsal part of the dorsal raphe nuclei in both groups of rats studied (Fig. 6 and Fig. 7).

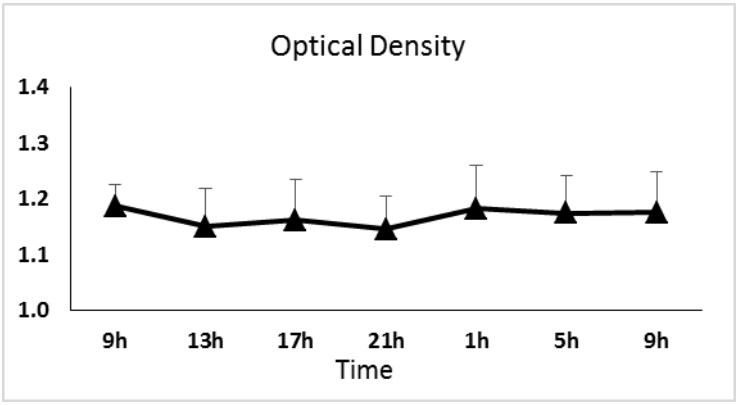

Figure 6: Daily changes in the concentrations of tryptophan hydroxylase enzyme represented by the optical density in the DM section of the dorsal Raphe nuclei of rats housed in conditions of light and dark succession 12:12 (p> 0.05) The light period extends from 6 to 18 o'clock.

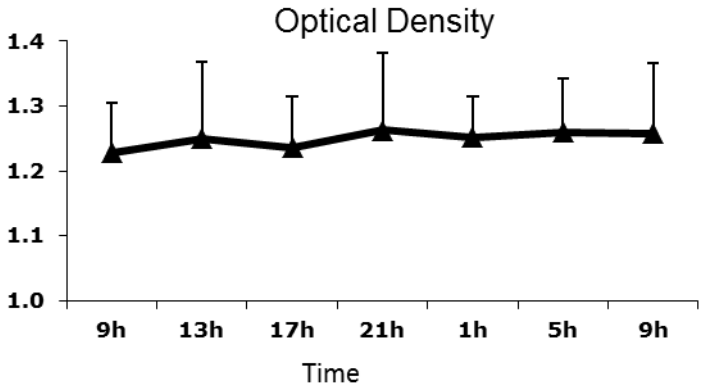

Figure 7: Daily changes in the concentrations of tryptophan hydroxylase enzyme represented by the optical density in the DM section of the dorsal Raphe nuclei of rats that were left in continuous dark conditions for 48 h before sampling (p> 0.05).

4- CONCLUSION

The results of this research point to the presence of clear, internal-origin circadian variations in the concentrations of tryptophan hydroxylase, the enzyme that determines the synthesis of the serotonin neurotransmitter, only in the lateral parts of the dorsal nuclei of the Raphe complex located in the midbrain. The fact that variations persist in constant dark conditions indicates that they are endogenous, and thus controlled by the SCN; the biological clock, and probably mediated by Glucocorticoids.[16,22-23] In a previous study, systemic changes in the Rapha nuclei tryptophan hydroxylase

mRNA content were totally abolished after the adrenal gland removal, and complete resetting was obtained using artificial Corticosterone surge.[16]

Regarding mRNA levels of tryptophan hydroxylase, a previous study showed that it had endogenous circadian systems, ie, under the influence of the biological clock of the SCN, throughout all the DR parts (VM, DM, L).[12] On the other hand, there is a systemic release of the neurotransmitter serotonin in both the biological clock SCN, and the thalamic Intergeniculate Leaflets (IGL), a key structure in the daily circadian system[24-27], which receive serotonin containing neurons from Dorsal Raphe, and particularly originating from the lateral parts.[6,8]

This explains the presence of tryptophan hydroxylase daily variations in only the lateral parts and the absence of other parts that do not directly interfere with communication with the components of the daily system. The gene of tryptophan hydroxylase can have a circadian expression at the level of the mRNA, then regulatory mechanisms may occur before the translation of RNA into a protein so that the variations become limited in the anatomically related parts of the circadian components, the lateral L parts as we have found, and the MR nucleus as demonstrated in previous studies.[12-14]

5- REFERENCES

1. Lucki, I., 1998. The spectrum of behaviors influenced by serotonin. Biol Psychiatry, 44: 151–162.
2. Salomon, R.M., Cowan, R.L., 2013. Oscillatory serotonin function in depression. Synapse, 67(11): 801-20.
3. Weinstein, J.J., Rogers, B.P., Taylor, W.D., Boyd, B.D., Cowan, R.L., Shelton, K.M., Salomon, R.M., 2015. Effects of acute tryptophan depletion on raphé functional connectivity in depression. Psychiatry Res., 2015 Nov 30; 234(2): 164-71.
4. Azmitia, E.C., Segal, M., 1978. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J Comp Neurol, 179: 641–667.
5. Hay-Schmidt, A., Vrang, N., Larsen P.J., Mikkelsen, J.D., 2003. Projections from the raphe nuclei to the suprachiasmatic nucleus of the rat. J Chem Neuroanat, 25: 293–310.
6. Meyer-Bernstein, E.L., Morin, L.P., 1996. Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation. J Neurosci, 16: 2097–2111.
7. Moga, M.M., Moore, R.Y., 1997. Organization of neural inputs to the suprachiasmatic nucleus in the rat. J Comp Neurol, 389: 508–534.
8. Villar, M.J., Vitale, M.L., Hokfelt, T., Verhofstad, A.A., 1988. Dorsal raphe serotoninergic branching neurons projecting both to the lateral geniculate body and superior colliculus: a combined retrograde tracing-immunohistochemical study in the rat. J Comp Neurol, 277: 126 –140.
9. Heyde, I., Kiehn, J.T., Oster, H., 2017. Mutual influence of sleep and circadian clocks on physiology and cognition. Free Radic Biol Med., S0891-5849(17): 31195-4.
10. Morin, L.P., 2013. Neuroanatomy of the extended circadian rhythm system. Exp Neurol, 243: 4-20.
11. Pilorz, V., Helfrich-Förster, C., Oster, H., 2018. The role of the circadian clock system in physiology. flugers Arch., 470(2): 227-239.
12. Malek, Z.S., Dardente, H., Pevet, P., Raison, S., 2005. Tissue-specific expression of tryptophan hydroxylase mRNAs in the rat midbrain: anatomical evidence and daily profiles. Eur J Neurosci, 22: 895–901.
13. Malek, Z.S., Pevet, P., Raison, S., 2004. Circadian change in tryptophan hydroxylase protein levels within the rat intergeniculate leaflets and raphe nuclei. Neuroscience, 125: 749–758.
14. Barassin, S., Raison, S., Saboureau, M., Bienvenu, C., Maitre, M., Malan, A., Pevet, P., 2002. Circadian tryptophan hydroxylase levels and serotonin release in the suprachiasmatic nucleus of the rat. Eur J Neurosci, 15: 833–840.
15. Dudley, T.E., DiNardo, L.A., Glass, J.D., 1998. Endogenous regulation of serotonin release in the hamster suprachiasmatic nucleus. J Neurosci, 18: 5045–5052.
16. Malek, Z.S., Sage, D., Pévet, P., Raison, S., 2007. Daily rhythm of tryptophan hydroxylase-2 messenger ribonucleic acid within raphe neurons is induced by corticoid daily surge and modulated by enhanced locomotor activity. Endocrinology, 148(11): 5165-72.
17. Harfstrand, A., Fuxe, K., Cintra, A., Agnati, L.F., Zini, I., Wikstrom, A.C., Okret, S., Yu, Z.Y., Goldstein, M., Steinbusch, H., Verhofstad, A., Gustafsson, J.A., 1986. Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. Proc Natl Acad Sci USA, 83: 9779–9783.
18. Clark, M.S., Russo, A.F., 1997. Tissue-specific glucocorticoid regulation of tryptophan hydroxylase mRNA levels. Brain Res Mol Brain Res., 48: 346–354.
19. Weissmann, D., Labatut, R., Richard, F., Rousset, C., Pujol, J.F., 1989. Direct transfer into nitrocellulose and quantitative radioautographic anatomical determination of brain tyrosine hydroxylase protein concentration. J Neurochem, 53: 793–799.
20. Weissmann, D., Chamba, G., Debure, L., Rousset, C., Richard, F., Maitre, M., Pujol, J.F., 1990. Variation of tryptophan-5-hydroxylase concentration in the rat raphe dorsalis nucleus after p-chlorophenylalanine administration: II. Anatomical distribution of the tryptophan-5-hy- droxylase protein and regional variation of its turnover rate. Brain Res., 536: 46–55.
21. Cash, C.D., Vayer, P., Mandel, P., Maitre, M., 1985. Tryptophan 5-hydroxylase: rapid purification from

whole rat brain and production of a specific antiserum. Eur J Biochem, 149: 239–245.
22. Spencer, R.L., Chun, L.E., Hartsock, M.J., Woodruff, E.R., 2017. Glucocorticoid hormones are both a major circadian signal and major stress signal: How this shared signal contributes to a dynamic relationship between the circadian and stress systems. Front Neuroendocrinol, S0091-3022(17): 30103-6.
23. Donner, N.C., Montoya, C.D., Lukkes, J.L., Lowry, C.A., 2012. Chronic non-invasive corticosterone administration abolishes the diurnal pattern of tph2 expression. Psychoneuroendocrinology, 37(5): 645-61.
24. Card, J.P., Moore, R.Y., 1989. Organization of lateral geniculate-hypothalamic connections in the rat. J Comp Neurol, 284: 135–147.
25. Challet, E., Pevet, P., Malan, A., 1996. Intergeniculate leaflet lesion and daily rhythms in food-restricted rats fed during daytime. Neurosci Lett., 216: 214–218.
26. Moore, R.Y., Card, J.P., 1994. Intergeniculate leaflet: an anatomically and functionally distinct subdivision of the lateral geniculate complex. J Comp Neurol, 344: 403–430.
27. Smith, V.M., Jeffers, R.T., Antle, M.C., 2015. Serotonergic enhancement of circadian responses to light: role of the raphe and intergeniculate leaflet. Eur J Neurosci, 42(10): 2805-17.